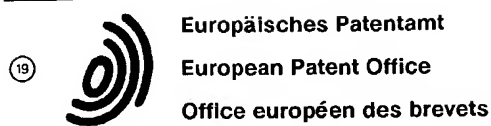


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54 **Anti-metastatic vaccine.**

57 In accordance with the present invention, there is provided an antitumor cellular vaccine comprising tumor cells into which *c-fos* gene alone or together with *c-jun* have been inserted.

Additionally, the present invention provides a method of treating a patient suffering from cancer to prevent and/or inhibit the development of metastasis which comprises the administration of the above mentioned cellular vaccine.

Further, the present invention provides an antitumor vaccine comprising antigens expressed by *c-fos* gene alone or together with *c-jun* gene.

EP 0 599 077 A2

The present invention relates to anti-tumor cellular vaccines and more specifically to vaccines for providing immunotherapy by gene therapy to specifically control the generation of metastases.

Generally, there is a correlation between the expression of particular genes and HLA antigens as well as a correlation between the presence of certain tumor associated antigens (TAA) antigens and metastasis.

However, there has been a problem in linking genetic control to control of metastasis and its inhibitions.

By way of background, malignant tumors arise from a protracted sequence of events. Each step in the sequence of events creates an additional phenotypic aberration (1). Research indicates that tumor cells acquire the ability to metastasize through genetic variation (2). Expression or loss of expression of specific genes has been associated with the metastatic phenotype of various cell lines (3,4).

Under normal conditions, the metastatic cells are subjected to attack by the host defense system, as long as the host defense system is competent to do so. An effective immune response capable of eliminating dissemination of tumor cells is predominantly mediated by cytotoxic T lymphocytes (CTL), which recognize proteolytically derived, foreign peptide epitopes bound to class I antigens of the major histocompatibility complex (MHC class I).

The inventors of the present invention reported that down regulation of MHC class I antigen was correlated with high malignancy in human and murine tumors (5). Transfection of MHC class I genes, particularly of H-2K genes was shown to confer on these tumors high immunogenic and low metastatic phenotypes.

Applicants previously found by testing high and low metastatic tumor cell populations that there was a correlation between the relative expression of class I antigens on different clones of a malignant carcinoma and the expression of the *c-fos* protooncogene (6,7). Although the involvement of oncogenes in the cellular transformation and tumorigenesis is apparently well established, the connection between oncogene or protooncogene expression and the metastatic competence of tumor cells remains unclear. Hence, use of this system clinically has not yet been perfected.

Studies by applicants suggest that the *c-fos* protooncogene participates in the control of MHC gene expression as evidenced by the following three studies. First, the *c-fos* gene was expressed in cells of the low metastatic clones of the 3LL tumor at much higher levels, for both *c-fos* transcripts and proteins, relative to cells of the high metastatic clones of the 3LL tumor (7). When the high metastatic clones were treated by interferon, a transient elevation of *c-fos* expression was observed followed by induction of H-2 transcription and there was a quantitative correlation between *c-fos* and H-2 induction (7,8). Second, a temporal correlation was found between the expression of the MHC class I antigens and the expression of *c-fos* antigens in a number of differentiating leukemic cells (9). Human U937 histiocytic lymphoma cells and HL60 promyelocytic leukemia cells, induced to differentiate to macrophages by 12-*O*-tetradecanoylphorbol 13-acetate (TPA), show induction of *c-fos* and HLA expression (9). In murine erythroleukemic cells, dimethylsulfoxide induced a decline in constitutive *c-fos* levels that were accompanied by suppression of MHC expression (9). Thirdly, transfection with *c-fos* genes of the clone D122 (3LL carcinoma), was shown to induce the transcription of H-2K mRNA and to elevate the levels of H-2 proteins, but not some of the other gene products (7,8).

Of critical importance is the finding that the rapid and transient induction of *c-fos* following cell stimulation by different external signals has established *c-fos* as a key member of the immediate early gene family and has implicated Fos in signal transduction and the control of cell proliferation (11,12) as well as in cell differentiation (13). Moreover, the Fos gene acts as a transcriptional regulator whose function is dependent upon formation of heterodimeric complexes with members of the *jun* family of protooncogenes (*c-jun*, *junB*, and *junD*) and further it binds to AP-1 consensus sequences, to CREB sequences and to Ap1/CREB like variations in the regulatory regions of target genes (12,14,15,16).

Applicant herein provides evidence of control of the *c-fos* and the unexpected results obtained thereby, and in combination with *c-jun*, control and/or inhibit metastasis in human tumors and murine models which are strongly predictive of human response. Based on experimental evidence herein relating to human tumor cells and HLA genes, applicant has determined the ability of the transfection and expression of particular genes to cause a dramatic decrease of tumorigenic and metastatic properties of various human tumors. Accordingly, applicant has derived an antitumor cellular vaccine and also a subcellular vaccine more specifically based on specifically expressed antigens for controlling tumorigenicity and metastatic properties of human tumor cells.

In accordance with the present invention, there is provided an antitumor cellular vaccine comprising tumor cells into which a *c-fos* gene (SEQ ID No:6) alone or together with *c-jun* gene (SEQ ID No:3) has been inserted.

Additionally, the antitumor cellular vaccine of the present invention is suitable for treating a patient suffering from cancer to prevent and/or inhibit the development of metastasis.

Further, the present invention provides an antitumor vaccine comprising antigens expressed by the *c-fos* gene alone or together with the *c-jun* gene.

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figures 1A-1D show expression of *c-fos*, *c-jun*, *junB* and H-2K^b: (A) in low (L) and high (H) metastatic clones of 3LL and K1735 tumors, (B) in D122 clones (3LL) transfected by *c-fos*, *c-jun*, or both, (C) in clone F10.9 (B16) transfected by *c-fos* and *c-jun*, and (D) in clone A9 (3LL) transfected by *junB*;

Figures 2A-2C show cell surface expression of MHC class I: (A) on clone D122 (3LL) and transfectants, (B) on clone F10.9 (B16) and transfectants, and (C) on clone A9 (3LL) and *junB* transfectants, where direct RIA were performed as described in experimental procedures and results are the means of five experiments, Standard errors were under 10% of the means;

Figures 3A-3D show regulation of MHC class I promoter activity by *c-fos*, *c-jun* and *junB*: (A) schematic drawing of the K^b enhancer - promoter domain -365 to 0 and some of the known binding elements, (B) D122 (-), J67 (*c-jun*), F6A2 (*c-fos*) and D13 (*c-fos + c-jun*) stable transfectants were transiently transfected by H2 promoter - CAT constructs, or (C) by collagenase - CAT constructs, (D) the A9 (-) and AJB1 (*junB*) stable transfectants, were transiently transfected by the H2 promoter - CAT constructs;

Figures 4A-4C show specific DNA binding activity of nuclear proteins from *c-fos/c-jun* transfectants, to the K^b enhancer A: (A) nuclear extracts from D122 and F10.9 transfectants, untreated (-) or treated (+) with cycloheximide were reacted at room temperature with enhancer A oligonucleotide and separated on acrylamide gel as described in experimental procedures, (B) reactions were performed as in A, in presence of 25 molar excess of competitor 'AP1', (C) nuclear extracts were incubated with the rabbit anti-*fos*- β -galactosidase serum (+) or anti H-2D^b nonrelevant antibodies (c) for 60 minutes at 4°C, probes were added and incubation was continued as before;

Figures 5A-5C show the effect of *c-fos* / *c-jun* transfection on tumorigenicity and metastasis, (A) D122 transfectants were injected i.f.p. into C57BL (A) or CD1 nude (B) mice, wherein foot diameters were measured as described in experimental procedures and the mean of tumor diameter of 16 mice in two experiments are shown, (C) D122, F10.9, A9 and transfectants were injected i.v. into C57BL mice;

Figures 6A-6D show experimental metastatic potential of D122, F10.9, A9 and transfectants: (A,B) D122, F6A2, J67, FJ23, D13, D6 and D36 clones, (C,D) F10.9, F21, F33, F32 and F52 clones wherein lungs were removed, weighed, fixed in Bouin solution and clarified in 70% ethanol; and

Figure 7 shows lytic activity of CTLs induced by D122 and transfectants on homologous target cells, C57BL mice were immunized with D122(-), J67 (*c-jun*), F6A2 (*c-fos*) and D13 (*c-fos + c-jun*) as described and EL4 (C57BL thymoma) and Yac-1 (an NK sensitive target) were used as control target cells, and were not lysed by any of the effector cells.

Generally, the present invention provides an antitumor vaccine which when injected into an animal produces stimulation of cytotoxic T lymphocytes (CTL) to produce an antitumorigenic and antimetastatic immune response. That is, the injection of the antitumor vaccine, whether in cellular or subcellular form, results in cytotoxic T cell lymphocyte activation which reduces or inhibits the generation of metastasis.

Specifically, the antitumor vaccine preferably is a cellular vaccine comprising tumor cells into which *c-fos* gene (SEQ ID No:6) alone, or the *c-fos* gene in combination with the *c-jun* gene (SEQ ID No:3), have been inserted. The tumor cells can be selected from a wide variety of tumor cells, preferably cells which can be efficiently and easily transfected with the above mentioned genes. For example, the cells can be carcinoma or melanoma cells, as well as other types of tumor cells. These cells can be obtained from the patient per se or can be obtained from a cell line not originally from the patient.

Tumor cells from the patients will be obtained either from biopsies, surgical material such as primary tumors, metastases in lymph nodes or other metastases, or in some cases from pleural effluents. Cell lines will be selected on the basis of the type of cancer: melanoma lines for melanoma patients, breast carcinoma lines for breast carcinoma patients, etc. The cell lines will be HLA matched. This means that HLA typing will be performed on each patient and cell lines which match in at least one HLA class I allele to the patient will be selected. If necessary a combination of cell lines will be used, each matching in at least one allele to the patient HLA. For example, a patient that is A1; A2; B7; B27; CW3; CW4 may get a vaccine consisting of six cell lines, each carrying one of these alleles. Additionally, if TAA typing is available, a vaccine that is also matched by TAA screening and HLA screening will be constructed. Preferably, the tumor cells are derived from tumor cells having metastatic competence, those cells being at least similar if not the same as the cells already existing in the patient. Such cells are rendered ametastatic by methods described below.

The means for inserting the *c-fos* gene or the *c-fos* gene together with the *c-jun* gene can be accomplished by methods known in the art. For example, the cellular vaccine can comprise human tumor cells transfected with either the *c-fos* gene alone or both *c-fos* gene and *c-jun* gene. For example, the two genes, the *c-fos* gene and the *c-jun* gene, can be introduced into the tumor cells on a single expression vector enabling constitutive production of the *c-fos* and *c-jun* gene products *in vivo*. Such methods have been previously detailed (7,23,44). Alternatively, the genes can be introduced into the tumor cells on different expression vectors enabling constitutive Production of the gene products *in vivo* by methods similar to those for the joint introduction, however, wherein each gene is transfected on an independent expression vector. Of course, consistent with the present invention, the *c-fos* gene alone can be introduced into the tumor cells on an expression vector.

A mammalian expression vector is a DNA construct that contains elements necessary for expression in mammalian cells, such as promoters, enhancers, termination and poly A signals, splice signals and of course a cDNA or genomic structure of the gene of interest. Such vectors may also contain sequences that enable them to replicate in bacteria, in which case the plasmid will contain bacterial replication signals and a gene for selection in a particular antibiotic such as an ampicillin-resistance gene.

Examples of expression vectors known in the art are genomic clones that contain autologous promoters and other processing signals (7); plasmid based vectors that do not carry a eukaryotic replicon, such as PTK2 (Wigeler, et al., 1977, Cell 11:223); PSV₂neo (Souther and Berg, 1982, J. Mol. Appl. Genet. 1:327); PRO-neo (Van Doren et al., 1984, J. Virol. 50:606); PSV₂gpt (Mulligan and Berg, 1980, Science, 209:1442), and others known in the art; plasmid DNA expression vectors containing regulatory sequences from eukaryotic viruses such as simian virus 40 vectors (Solnick, D., 1981, Cell 24:135), bovine papilloma virus based vectors (Sarver et al., 1981, Mol. Cell Biol. 1:486), Epstein-Barr virus based vectors (Yates et al., 1985 Nature 313:812). In addition Retroviral vectors (Gilboa et al., 1986, Biotechniques 4:504), Adenovirus (Berkner K.L., 1988, Biotechniques 6:616), and Vaccinia virus (Fuerst et al., 1987, Mol. Cell Biol. 7:2538) which are used as infectious particles but do not replicate in the recipient cell because of structural manipulations can be used.

With some expression vectors, such as retroviral vectors or adenovirus based vectors, the structure of the vector also suggests the method of gene transfer (infection). With other expression vectors, different gene transfer methods, which are the methods of introduction of DNA containing the expression vector into the target cells, are used. Examples of gene transfer methods are: calcium phosphate (40), Liposomes (46), DEAE Dextran (47), Polybrene (48), Protoplast fusion (49), and others known in the art.

In each of the above mentioned methods, gene transfer is performed before cell inactivation, if the cells are to be inactivated.

Preferably, the tumor cells used for the antitumor cellular vaccine are inactivated, although inactivation is not necessary in all instances. Inactivation of the tumor cells is preferably performed by irradiation with gamma source at 3,000 to 10,000 Rad or by treatment with mitomycin C at concentrations of 30 to 100 µg/ml for one hour (50). An alternative is to utilize both treatments to insure inactivation of the tumor cells (51). Of course, other methods known in the art, such as fixation in glutaraldehyde (0.1 to 1%) for 5 to 30 minutes may also be used (52).

The *c-fos*, and *c-jun* genes are preferably cloned from cDNA libraries as set forth below under the Methods section. The *c-fos* + *c-jun* fused plasmid is described below also. The preferred method of transfection is described below under "preparation of stable transfectants". However, alternative methods can be used such as using the *c-fos* (SEQ ID No:6) and *c-jun* (SEQ ID No:3), both of human origin under the control of various promoters, such as the viral long term repeats (LTR) of various origins, under the control of a β actin promoter, or under strong promoters of enzymes, such as DNA polymerase type III as known in the art (53).

Preferably, the vaccine is formulated as an injection. The vaccine can contain inactivated tumor cells, inactivated as described above, in a saline or phosphate buffered saline. Preferably, no adjuvants would probably be required although alternative methods of preparation can include adjuvants such as BCG (54) or Alum (55), may be considered for use. Intactness of cells may be important for antigen presentation and cytokine release. Adjuvants usually cause cell lysis, for example Freund's complete adjuvant (56).

Preferably, the vaccine would include 1×10^6 to about 1×10^9 transfected tumor cells. Such dosages in human patients would be monitored either by skin tests or by reactivity of lymphocytes in one of the following methods: 1) nontransfected and transfected cells after inactivation are intradermally injected into the thigh or upper arm of the patient and DTH activity is measured by diameter of both the erythema and induration at 24 and 48 hours. Such instances would normally be negative. The patient is vaccinated and retested every two to four weeks. Maximal DTH is an indication for sufficient vaccination. 2) Blood can be drawn from the patient before vaccination. White blood cells are separated and viably frozen. Similar

samples are taken at various time points after vaccination and booster injections. The ability of the white blood cells from different stages of vaccination to lyse nontransfected and transfected cells is measured in a CTL (cytotoxic T-lymphocyte) assay (57). Stabilization of the lytic activity is a measure for maximum vaccination.

5 The antitumor vaccine made in accordance with the present invention need not necessarily be a cellular vaccine, what is critical is that the vaccine include immunogenically competent HLA antigens derived from the expression of the *c-fos* gene alone or together with the *c-jun* gene and immunogenic tumor associated antigen (TAA) derived peptides. Such preparations can be made from cell membrane preparations of the transfected tumor cells. The difficulty of this approach is that such membrane preparations can
10 hide the antigens depending upon the folding of the membranes. However, such vaccines have been made in the art for other preparations (58).

The vaccines of the present invention are suitable for the treatment of a patient suffering from cancer, the treatment preventing and/or inhibiting the development of metastasis.

Generally, this treatment includes a step of administering to the patient the vaccine described above.
15 More specifically, the patient would be treated as follows. First, cells from the primary tumor of the patient would be removed by biopsy or surgery. As discussed above, an alternative approach would be to use cells derived from other patients. In either case, the cells would be dispersed in a medium, such as Dulbecco's modified eagle medium (DMEM), RPMI or other mediums used for the preparation of cellular vaccines. A vector, such as the vectors discussed above, is inserted into the cells. The vector would comprise the
20 human *c-fos* gene or the human *c-fos* and *c-jun* genes. During this step, the positive transfectants that are high expressers of *c-fos* and MHC class I genes can be selected either by an appropriate antibiotic (G418 if a neo resistant gene is present on the vector, (59) or by binding of anti-HLA antibodies and enrichment by a Fluorescence Activated Cell Sorter (FACS). The transfectants are inactivated as described above by gamma or x-ray irradiation and/or treatment with mitomycin C as described above. Finally, an
25 effective amount of inactivated tumor *c-fos* or *c-fos* and *c-jun* transfected cells is administered into the patient, preferably by injection. A highly immunogenic vaccine is thus obtained for preventing and/or inhibiting tumor metastasis in the patient.

The following experimental evidence provides a basis for utilization of the present invention and its effectiveness in decreasing and/or preventing metastasis. Further, experimental evidence demonstrates the
30 mechanism of action by which cytotoxic T lymphocytes (CTL) are activated to produce the immunogenic response.

EXPERIMENTAL PROCEDURES

35 TUMOR CELLS

Tumor cells were maintained in DMEM, 10% fetal calf serum, and supplements (6). The Lewis lung carcinoma (3LL) and the B16 melanoma (B16) are malignant tumors which originated spontaneously in C57BL/6 (H-2^b) mice (17,18). A9 and D122 are low and high metastatic clones, respectively, cloned from
40 the 3LL carcinoma cells by limiting dilution (6). The high metastatic line B16-F10 was selected from B16 by I.J. Fidler (17). F10.9 is a high metastatic single cell clone derived from the B16-F10 line (19). The primary K1735 melanoma arose in an inbred C3H/HeN mouse following a short exposure to U.V. irradiation, it was transplanted once into an immunosuppressed recipient and then established in culture (20). The low-
45 metastatic line 16 and the high metastatic line M4 were selected from K1735 by I.J. Fidler (20).

ASSAYS FOR TUMORIGENICITY AND METASTASIS

C57BL/6J (Jackson Laboratories, Bar Harbor, Maine) or CD1 nude mice (Weizmann Institute Breeding Center) were injected with 2×10^5 3LL or B16 tumor cells and C3H/HeN (Jackson Laboratories, Bar Harbor,
50 Maine) mice were injected with 2×10^5 K1735 tumor cells, intrafootpad (i.f.p.) in the right hindleg. To monitor tumor growth, the diameter of tumor bearing feet were measured every 1-3 days with calipers. When tumor diameter reached 8 mm, the tumor bearing foot was amputated. Survival and formation of spontaneous lung metastases was monitored at the time that mice became moribund. Experimental lung metastases were tested by injecting C57BL/6J or CD1 nude mice with 5×10^5 3LL or 5×10^4 B16 tumor cells, and C3H/HeN
55 mice with 5×10^5 K1735 tumor cells, into the mouse tail vein. Mice were sacrificed 30-35 days later for D122, F10.9 and M4 (high metastatic) clones, and 65-70 days later for A9 (low metastatic) clones, and lungs were excised and weighted. Lung weights are the averages of three experiments. Maintenance and experimental procedures of mice were performed according to NIH guidelines.

GENE EXPRESSION

5 RNAs were prepared from $1-3 \times 10^8$ cells propagated in tissue culture or treated with $10 \mu\text{g/ml}$ of cycloheximide for one hour, by the method of Chirgwin (21). Northern blots were prepared from formaldehyde-containing agarose gels loaded with $30 \mu\text{g}$ total RNA per lane as described (7) and assayed by hybridization to [^{32}P] labeled probes. The following probes were used: *c-fos* - 1.2 kbp HindIII-EcoRI fragment from the pBK28 fos plasmid (22), *c-jun* - 1.1 kbp EcoRI-PstI fragment from chJ-2 plasmid (23), *junB* - 1.2 kbp HindIII-Nde I fragment from RSVjunB plasmid (24), for H-2K - the H-2K specific 30-mer oligonucleotide which codes for amino acids 148-157 of the H-2K^b protein. This oligonucleotide crossreacts with H-2K^k (26/30 nucleotides). For the β actin probe, a 4.3 kbp insert of pAC18.1 was used (25).
 10 Hybridizations were performed in 50% formamide at 42° . Blots were washed in 0.1SSC-0.2% SDS at 60° . Blots hybridized to oligonucleotide probes were washed in 0.5 SSC-0.2% SDS at 50° .

PLASMIDS AND CONSTRUCTS

15 The plasmids Psv-cJun (23), pBK28 (22) and RSVjunB (24) have been previously described and characterized by others. *c-fos* + *c-jun* fused plasmid (CON. 9) was constructed by ligation of a HindIII-NdeI fragment from Psv-cjun (containing SV40 early promoter, *c-jun* cDNA and SV40 polyadenylation signal) into HindIII linearized pBK28, *c-fos* expression vector, blunt ending and religation. The *c-jun* and *c-fos* are transcribed from separate promoters (SV40 and LTR, respectively) and the construct contains two poly A addition signals. PUC-365-CAT was prepared by cloning of a BamHI-XbaI restriction fragment from pH-2CAT (39) into a Puc19 vector. PUC-142-CAT and PUC-190-CAT were subcloned from p138H-2K CAT and p190H-2K CAT, respectively, into Puc19 vector (26), p38-H-2K-CAT, the collagenase promoter-CAT constructs, -73COL-CAT and -60COL-CAT (32) and the β galactosidase containing expression vector PCH110 (Pharmacia, Inc.) were described before by others.
 25

PREPARATION OF STABLE TRANSFECTANTS

30 Transfections of *c-jun*, *junB*, *c-fos* or *c-fos* + *c-jun* (CON.9) expression vectors into the high metastatic clone D122 (3LL), transfection of *c-fos* + *c-jun* (CON.9) into the high metastatic clone F10.9 (B16) and the transfection of *junB* into the low metastatic clone A9 (3LL) were performed by the calcium phosphate technique, in cotransfection with a 1:9 or 1:19 ratio of PSV₂neomycin resistance gene (PSV₂neo) (40). Control transfection was done with PSV₂neo alone. To increase the efficiency of transfection, a 10 minute treatment with DMEM-15% DMSO (dimethylsulfoxide) was performed after incubation with DNA precipitates. Colonies growing in $400 \mu\text{g/ml}$ G418 (GIBCO) four weeks after transfection were expanded and analyzed for expression of the inserted genes by Northern blot analysis as described above. The selected D122 *c-fos* high expressor F6A3 clone was further supertransfected by the *c-jun* expression vector, in cotransfection with 1:9 ratio of pSV2 hygromycin B resistance plasmid (pSV2hygro) and the selection was done in $200 \mu\text{g/ml}$ hygromycin B containing media. Control transfectants carrying pSV₂neo or pSV₂hygro show hybridization patterns, MHC class I expression and malignant properties equal to parental cells. (19)
 40

CELL-SURFACE H-2 ANTIGENS

45 Protein A-purified monoclonal antibodies 20-8-4 (αK^b) and 28-14-8 (αD^b) (27) were iodinated by chloramine T by standard protocols. Five hundred ng of labeled antibody were mixed with 5×10^5 freshly trypsinized cells in 0.1 ml phosphate-buffered saline (PBS) in BSA-coated tubes. Triplicate samples were incubated at 0° for 90 minutes. After four washings in PBS-0.5% bovine serum albumin (BSA) 0.02% sodium azide, samples were monitored in a gamma scintillation counter.

TRANSIENT EXPRESSION ASSAYS

55 The Chloramphenicol acetyl transferase (CAT) gene codes for an enzyme that transfers a labeled ^{14}C acetyl or butyryl group from [^{14}C] acetyl CoA or [^{14}C] butyryl CoA to chloramphenicol. It serves as a "reporter gene": when the structural CAT gene is ligated to promoter sequences of various genes and these constructs are transfected (transiently or stably) into cells, one can measure the CAT activity in cell lysates and learn from the data about the activity of the promoter fused to CAT in the particular cell lines. There is almost no background in mammalian cells since CAT is a bacterial enzyme (41).

For transient assays, 1×10^6 cells were plated in 10cm dishes, 24 hours before DNA transfection. Cells were incubated for 12 hours with calcium phosphate-precipitated plasmid DNAs (20 μ g of the CAT derivative plus 4 μ g of pCH110) then, following a 10 minute DMSO (15%, 37°C) shock, the cells were rinsed once, reseeded with fresh medium and 24-48 hours later the cells were processed for enzymatic assays. CAT activity, using 30 μ g of total cell extract protein, was determined as described (41), and β -galactosidase activity was determined as described (42). Experiments were repeated at least three times. Activity of CAT was normalized to activity of β -galactosidase to correct for difference in transfection efficiency.

GEL RETARDATION ASSAY

10

Nuclear extracts were prepared from 10×10^6 cells and used in gel retardation assays as described (28). Protein concentration were determined using the Bradford method (60) and ranged from 4-7mg/ml. A synthetic double stranded 49 bp oligodeoxynucleotide containing the entire enhancer A was used as a probe. The sequence (SEQ ID No:8) of the enhancer A fragment is:

15

GGCAGTGAGGTCAGGGGTGGGGAAGCCAGGGCTGGGGATTCCCCATCT
GTCACCTCCAGTCCCCACCCCTTCGGGTCCCGACCCCTAAGGGGTAGAGG
-205-154

20

The AP1/CREB like nonlabeled competitor oligonucleotide was used in the binding assay (indicated by the broken line above domain -205 to -186 containing the AP1 like binding site in enhancer A (from -203 to -197). A nonrelevant 19-mer nonlabeled competitor oligonucleotide was used as a control.

25

DNA-protein binding was conducted in 20 μ l volumes. The nuclear extract (3-5 μ g) was incubated with 3 μ g of poly(dI•dC) (Pharmacia Inc.) for 15 minutes at room temperature. Approximately 0.1pmol of 32 P-labeled DNA (~10,000 cpm) was added to the preincubated nuclear extract. Unlabeled competitor DNA was added to the binding reaction two minutes before the labeled oligomer. Rabbit anti-*fos*- β -galactosidase fusion protein antiserum (43), when used, was added to the nuclear extracts for one hour at 4°C, prior to 32 P-labeled DNA addition. No disruption of the nucleoprotein complex binding was observed when a control antiserum was added to the extract. DNA-protein complexes were resolved on 4% polyacrylamide gel (39:1 acrylamide to bisacrylamide) in 0.4X TBE (1X TBE is 50 mM Tris-borate [pH 8.3] 1mM EDTA). The gels were dried and autoradiographed with intensifying screens at -70°C.

30

35 IN VITRO LYTIC ACTIVITY (CTL) ASSAY

C57BL/6J mice were immunized intraperitoneal (i.p), three times at 7-day intervals, with 2×10^6 tumor cells, irradiated (5000 Rad) and mitomycin C treated (80 μ g/ml/ 5×10^6 cells). Spleen cells were removed 10 days after the last immunization and restimulated *in vitro* for 5 days with irradiated cells (as before) at ratio of 20:1 (responders/stimulators). Spleen cells were seeded at 4×10^6 cells/ml, in RPMI medium containing 10% FCS, 0.4% combined antibiotics, 2 mM glutamine, 1mM sodium pyruvate, 1mM nonessential amino acids, 2×10^{-5} M β -mercaptoethanol and 10 mM Hepes pH 7.4. On day 5, stimulated spleen cells were separated on lymphocyte preparation medium (Cedarlane, Ontario), washed three times with PBS and suspended at a concentration of 5×10^6 cells/ml. Five thousand target cells labeled with 35 S-methionine (NEN) for 6 hours were suspended in 96 u-shaped wells and graded numbers of effector cells were added at E/T ratios of 100:1, 50:1, 25:1 and 12.5:1. Plates were incubated for 16 hours for D122 and *fos/jun* transfectant targets, and 5 hours for EL4 and Yac1 targets at 37°C, 5% CO₂. Microplates were spun at 1000 rpm for 10 minutes and 100- μ l aliquots of supernatant were transferred into tubes, mixed with 3 ml scintillation fluid and monitored in a beta counter. Spontaneous release was determined by incubation of labeled cells with medium alone; maximal counts were determined by incubating the same target cells with 100 μ l 0.1 NaOH. Spontaneous release was below 20% of maximal release. Specific release is reported as the mean of triplicates values.

50

55

RESULTS OF EXPERIMENTATION

EXPRESSION OF *c-fos*, *c-jun*, *junB* AND H-2K GENES IN MURINE TUMORS.

Low metastatic 3LL clones were characterized as high expressors of *c-fos* and MHC class I genes in contrast to high metastatic 3LL clones that express low levels of both genes (7,29). To determine whether this correlation could be extended to other murine tumor systems the steady state mRNA expression of *c-jun*, *junB*, *c-fos* and H-2K (MHC) genes was assayed in K1735 melanoma (30) and B16 melanoma (17) metastatic tumors as well as in the Lewis lung carcinoma (3LL).

Northern blot analysis of the high metastatic clones D122 (3LL), M4 (K1735) and F10.9 (B16), and the low metastatic clones A9 (3LL) and 16 (K1735) is shown in Figure 1 (A and C). Hybridization was performed at 42°C in 50% formamide and 10% dextran sulfate for 36 hours. Blots were washed in 0.1 SSC, 0.1% SDS at 60°C. Hybridization to β actin probe was included as a measure for equal levels of RNA in the samples. The typical 2.2 kb *c-fos*, 2.7 kb *c-jun* and 2.0 kb *junB* transcripts appear in high metastatic D122 cells and at much higher levels in A9 (3LL) low metastatic cells (Figure 1A lanes 1-2). Thus the correlation observed before (6,7) between low metastatic potential and elevated expression of *c-fos* and H-2K is also observed for *c-jun* and to a lesser extent for *junB* expression. Cells of the low metastatic clone 16 (K1735) expressed high levels of the *c-jun*, *junB*, *c-fos* and H-2K transcripts (Figure 1A lane 4), while only minor levels of these transcripts were observed in cells of the high metastatic clones, M4 (K1735) (Figure 1A lane 3) and F10.9 (B16) (Figure 1C lane 1). Several variants of the presumably low metastatic B16 line, F1 that were tested, were all high metastatic *in vivo* and show expression profiles similar to F10.9. Thus no direct correlation can be shown in the B16 tumor.

The differential expression of *junB* and *c-jun* in K1735 clones did not change after a cycloheximide (CHX) treatment, which is known to stabilize and superinduce *c-fos* and *c-jun* mRNA's (31). CHX treatment caused a significant induction of *c-jun* and *junB* mRNA's in clone 16 cells, but only a marginal elevation in the M4 cells (Fig. 1A panel 2 and 3, lanes 5-6). In accordance with H-2K mRNA levels, clone 16 cells manifested high levels of H-2K^k cell surface glycoproteins, as opposed to almost complete lack of H-2K^k antigens on the cell surface of M4 cells (data not shown). The expression of H-2D^k was similar between the two clones (data not shown).

EFFECT OF *c-jun*, *junB* AND *c-fos* TRANSFECTION ON EXOGENOUS AND ENDOGENOUS GENE EXPRESSION.

The correlation between *c-fos* and *c-jun* gene expression and the low metastatic phenotype observed in 3LL carcinoma and in K1735 melanoma clones, raised questions concerning the cause-effect relation between these phenomena. To investigate the possibility that *c-fos* + *c-jun* family of genes play a role in regulating MHC class I expression, low K^b expressing D122 (3LL) cells were transfected with *c-jun*, *junB*, *c-fos* or *c-jun* + *c-fos* plasmids. Plasmids Psv-c-jun which contain the mouse *c-jun* cDNA (SEQ ID No:1) under SV40 early promoter control, RSV-JunB which contains the mouse *JunB* cDNA under an LTR control and pBK28 which contains the human *c-fos* (SEQ ID No:6) cDNA under *v-fos* promoter control were used. Cotransfection was carried out with PSV₂neo cDNA at 9:1 ratio and selection in neomycin (G418) followed.

The *c-jun* + *c-fos* transfections were done either by supertransfection of a *c-fos* positive D122 clone (F6A3), with *c-jun* and hygromycin-B resistance (psV2hygro) plasmids or by transfection of a *c-jun* + *c-fos* expression construct *Con.9*, which assures that *c-fos* and *c-jun* transfected genes would be integrated in a similar copy number and in the same place in the cell genome. The low K^b, D^b expressor clone F10.9 (B16), was also transfected with the *c-fos* + *c-jun* plasmid *Con.9*. Figure 1B demonstrates the expression patterns of the various D122 stable transfectants and the effect on expression of endogenous genes of the *fos* and *jun* family as well as on the expression of the endogenous H-2K genes. Expression of endogenous *junB* is highly induced by *c-fos* and *c-jun* overexpression, as demonstrated on the Northern blots in Fig. 1B (panel 3). Interestingly the parental D122 cells express a 2.0 kb transcript while the *c-fos* and *c-jun* transfectants express a closely spaced *junB* mRNA doublet of 2.0 and 2.1 kb. This doublet is likely to represent the use of different polyadenylation sites (44). Notably, transfectants of *c-fos* + *c-jun* (clones FJ23 and D13) express less *junB* transcripts than transfectants of either *c-fos* or *c-jun* alone.

The positive transcriptional regulation of the *junB* gene, by *c-jun* and *c-fos* has not been described before. However, the promoter of *c-jun* was shown to contain an AP1 binding site and *c-jun* (probably by Jun-Fos complex) is an efficient activator of its own expression (32). Since the gene for *junB* is structurally

related to *c-jun* (33) it is conceivable that it might be similarly regulated.

c-fos transcript is overexpressed in *c-fos* and in *c-fos + c-jun* transfected clones (Fig. 1B panel 1). Since endogenous and exogenous transcripts are of a similar size, it is not clear whether the exogenous *c-fos* expression or *c-fos + c-jun* coexpression, actually reduced the endogenous *c-fos* expression. Unexpectedly *c-jun* transfected also show high levels of the 2.2 kb endogenous *c-fos* transcript (Fig. 1B panel 1).

Although repression of *c-fos* promoter, by the Fos protein has been demonstrated in NIH3T3 and HeLa cells (34), and this repression was enhanced by coexpression with *c-jun* (35), in several other cell systems, introductions of exogenous *c-fos* genes, did not down regulate the expression of the endogenous *c-fos* gene (36).

Moreover, introduction of *c-fos* into ES cells and production of chimeric *c-fos* mice showed that in these ES cells, in the chimeric mouse tissues and in tumor derived cell lines from these mice, both endogenous and exogenous *c-fos* RNA as well as *c-jun* RNA were highly elevated (37).

This may possibly be the result of the distinct cellular environment in the different cell lines. It has been previously suggested that the repression of *c-fos* is the result of *c-fos + c-jun* complex interaction with another cellular protein or alternatively Fos and Jun may act independently on the same or different target proteins (35). Thus, it may depend on the precise composition of the cellular proteins whether a repressing or an activating effect is achieved.

Increased levels of 2.7 kb endogenous *c-jun* transcripts are expressed in all *c-fos* transfected cells (Fig. 1B panel 2). Induction of *c-jun* in D122 cells transfected with human or mouse genomic *c-fos* were also observed (unpublished results). Fig. 1B panel 2 also shows *c-jun* overexpression in *c-jun* and double transfected cells. *c-jun* was shown to transactivate its own promoter (32), and in addition, the transfected *c-jun* cDNA (Psv-*c-jun*) is driven by the SV40 promoter, which contains two AP1 binding sites, and may also be positively regulated by *c-jun*. This can account for the very high mRNA *c-jun* observed in J67 and FJ23 cells.

To summarize, *c-fos* and *c-jun* transfections into D122 cells induced endogenous *junB* mRNA. In addition, *c-fos* or *c-jun* transfection resulted in a reciprocal transcriptional activation, namely, the *c-fos* transfected D122 cells showed increased levels of the endogenous *c-jun* transcripts and the *c-jun* transfected D122 cells showed an increase in the endogenous *c-fos* gene expression. A similar analysis of the RNA from *junB* transfected clones did not reveal significant changes in endogenous *c-fos* or *c-jun* expression compared to the parental D122 cells (data not shown).

ELEVATED MHC CLASS I IN *c-fos* and *c-jun* TRANSFECTANTS, REDUCED H-2 IN *junB* TRANSFECTANTS.

Hybridization of RNA extracted from the various *c-jun*, *c-fos* and double transfected cell lines and the parental D122 clone to an H-2K^b specific probe is shown at Fig. 1B panel 4. All the transfectants that expressed high levels of *c-fos* and *c-jun* mRNA show transcriptional activation of the 1.8 kb endogenous H-2K^b mRNA. The highest H-2K mRNA expressor among these transfectants is clone D13 which contains the fused *c-fos + c-jun* genes. Notably the D13 cells show the lowest levels of *junB* mRNA transfectants, 3 out of 12 *c-fos* transfectants and 11 out of 35 *c-fos + c-jun* double transfectants which did not express elevated *c-jun* and *c-fos* mRNA's, did not show activation of H-2K transcription (data not shown), while all *c-fos* and *c-jun* single and double positive clones showed elevated steady state levels of H-2 mRNA. The data shown in Figure 1C demonstrate similar results in F10.9 (B16 melanoma) cells, namely the positive stable F10.9 *c-jun + c-fos* transfected cell lines (F52 and F21) manifest a marked transcriptional activation of the H-2K^b gene relative to the parental, low expressor F10.9 clone (Fig. 1C panel 3). The H-2K^b mRNA expression pattern in D122, *junB* transfected clones remained very low, similarly to the parental D122 phenotype (data not shown). To evaluate the increase in H-2 related proteins in the *c-fos* and *c-jun* transfected D122 (3LL) and F10.9 (B16) clones, direct radioimmunoassays were performed using [¹²⁵I] labeled monoclonal antibodies to H-2K^b and H-2D^b antigens (27). Two to nine fold elevated levels of H-2K^b and 1.2-7 fold elevated H-2D^b expression on the cell surface of *c-fos*, *c-jun* and double transfected cells was observed (Fig. 2A and B).

These results indicate that MHC (H-2K and H-2D) expression from endogenous genes can be induced by *c-fos* and *c-jun* transfection, either by single gene transfer or by cotransfection with both genes. In contrast, the D122 *junB* transfected clones show a decrease in the H-2^b cell surface expression (data not shown).

To further examine the possibility that *junB* might down regulate MHC class I expression, the *junB* gene was transfected into the high H-2K^b, H-2D^b expressor A9 (3LL) clone. Figure 1D shows the hybridization of

RNA extracted from the highest *junB* expressor clone AJB1 and parental A9 clone cells to the specific *junB*, *c-jun*, *c-fos* and H-2K probes. Most of the exogenously expressed JunB seems to appear as a 2.1 kb transcript in a closely spaced *junB* mRNA doublet of 2.0 kb and 2.1 kb while the endogenous *junB* transcript is primarily of the 2.0 kb (Fig. 1D, panel 1). The H-2K hybridization of pattern (Fig. 1D panel 2) indicates a decrease in the H-2K^b steady state mRNA levels in the transfected cells, relative to the parental high H-2K^b expressor A9 cells.

No significant changes were observed in *c-fos* and *c-jun* expression following the *junB* transfection (Fig. 1D, panels 3 and 4). Hybridization to β actin probe showed that equal amounts of RNA were used (not shown). Figure 2C demonstrates the cell surface expression of H-2K and H-2D proteins in the *junB* transfected clones. A decrease of 40%-70% below control (A9) is observed for the H-2K^b molecules and of 10%-40% for the H-2D^b molecules in A9 *junB* transfectants (Fig. 2C).

ACTIVATION OF THE H-2 PROMOTER BY *c-fos* AND *c-jun*

To address the possible mechanism by which cJun, JunB and cFos proteins regulate H-2 expression, portions of the H-2K promoter were fused to the CAT gene and activities were monitored following transient transfections into the stable *jun(s)* and *c-fos* overexpressing cells.

The plasmids PUC-365-CAT, PUC-190-CAT, PUC-142-CAT and p38-H-2K-CAT (p365, p190, p142, and p38 in Fig. 3) were used which are different deletion constructs of the 5' flanking region from the protein cap site, fused to the CAT gene (see fig. 3A). At least three separate assays were performed with each transfect as described in experimental procedures. Kinetics between 1-30 hours were monitored. Enzyme activities are linear up to 20 hours. Activities at 15 hours, of one representative assay are described. The construct PCH110 (containing the β -galactosidase gene fused to the SV40 promoter) was cotransfected with each of the CAT plasmids as an internal control to transfection efficiency.

As shown in Figure 3B high cJun, cFos expression of the transfected cells resulted in a marked activation of the p365 construct. The activation magnitude correlated well with the induction of H-2K mRNA, (Fig. 1B), and with the increase in H-2 related proteins on the cell surface (Fig. 2A) of the transfected clones. In contrast, transfection with p142 that contains enhancer B only (Fig. 3B) or with p38 that contains only the TATA box (data not shown), into parental D122 or *c-fos*, *c-jun* transfectants, yielded very low CAT activity. The lower activation of p190 compared to the activation of p365 in *c-fos* and/or *c-jun* expressor cells (Fig. 3B), indicates that the AP1 like binding sequence (-200 -193) plays a major role in the activation of H-2 by the cFos and cJun proteins. Yet the activation of the p190 construct in cJun and cFos/cJun expressors, relative to the nonexpressor D122, indicates that the domain downstream to -190 is also regulated by *c-jun* or *c-jun* + *c-fos*. This cannot be attributed to the AP1-like domain in enhancer B since the p142 construct is hardly active in all lines (Fig. 3B).

These data suggest that the region between -190 and -142 includes an additional target of activation by the Fos-Jun complex which is not directly mediated by an AP1-sites in the promoter, since the sequence between -190 -142 does not contain an obvious AP1 binding site. It is possible that another gene product that is regulated via TRE/AP1 is a regulator of the IRS, or of the NF κ B binding domain (see Fig. 3A) or that the cJun or cFos proteins interact directly with one of the nuclear proteins that bind in the -190 -142 promoter domain (see also reference 38).

To verify that a classical AP1 enhancer is also activated in these cells, the constructs -73Col•CAT and -60Col•CAT were used which contain the collagenase promoter region from position -73 to +63 or -60 to +63 respectively, fused to CAT gene at position +63 (32). The two constructs differ from each other by the deletion of the AP1 sequence between -73 and -60 in the collagenase promoter. The basal level of CAT expression from these constructs in the absence of appropriate stimulating factors is usually very low. The CAT expression following transient transfections of -73Col•CAT (Fig. 3C) resembled the expression patterns observed with the p365 construct of the H-2 promoter (Fig. 3C), namely, exogenous cFos and cJun expressors stimulated expression from either promoters, relative to the basal D122 activity. The AP1 deletion mutant -60Col•CAT (Fig. 3C) showed a low activity in Fos and Jun expressor cells, similar to the activity in parental D122 cells.

PROTEIN BINDING TO ENHANCER A IN *c-fos/c-jun* TRANSFECTANTS.

Gel mobility shift assays were performed to analyze the binding properties of nuclear proteins from *c-fos* and *c-jun* overexpressing cells, to the oligodeoxynucleotide sequence of the entire enhancer A region (see Fig. 3A and experimental procedures). As shown by Figure 4A, extracts from the D122 cells show formation of four complexes (bands I-IV) with enhancer A. CHX treatment induces mostly band I. D122

transfection with *c-fos* alone, *c-jun* alone or both, shows elevated formation of complexes I and IV, and to a lesser extent of band II, as well as a decrease of the intensity of band III (Fig. 4A,B). The non-labeled 'AP1' oligonucleotide (that contains AP1/CREB and flanking AP2 sequences, see experimental procedures) competes with bands I-III, and to a lesser extent with band IV (Fig. 4B).

5 Formation of complexes III and IV seems to be temperature dependent, since preincubation of nuclear extract with anti-Fos antibody or a control antibody at 4°C for one hour prior to addition of the labeled probe decreased significantly bands III and IV. In addition, under these conditions band II seems to split into two distinct complexes, II and II' (Fig. 4C). Complex I, which is elevated by *c-fos/c-jun* transfection, is specifically inhibited by pretreatment of the nuclear extracts with anti-Fos antibodies, but not with an
10 unrelated, cell surface antibody (anti H-2D^b). Complex II that is marginally affected by the *c-fos/c-jun* transfection, seems also to be inhibited by anti-Fos, while complex II' is not.

Nuclear complex profiles of F10.9(B16) melanoma cells, show quantitatively a different profile; complex I, II, and IV are hardly visible, and band III constitutes the major complex. Upon CHX treatment bands I and II are intensified. Again, transfection of *c-fos+c-jun* elevated complexes I, II and IV, and reduced
15 complex III (Fig. 4A). These experiments indicate that transfection with *c-fos/c-jun* or both, increase the binding of specific complexes to enhancer A, and that the Fos protein is a part of at least two of these complexes (I and II).

NEGATIVE REGULATION OF H-2 BY JunB DOES NOT REQUIRE THE AP1 BINDING SITE

20

The repression of H-2 cell surface expression of *JunB* transfectants (Fig. 2C) was well correlated with the reduced levels of H-2K mRNA (Fig. 1D). To delineate the target of the JunB negative regulation on H-2 promoter transient transfection of H-2 CAT constructs was performed into the constitutively *JunB* overexpressing cells (Fig. 2C). Fig. 3D shows that construct p365 which has the maximal basal activity in A9 (3LL)
25 cells is repressed in the *JunB* expressor AJB1 cells to approximately 70% of the basal (A9) activity (Fig. 3D, compare lanes 1 and 2). The activity of p190, which is 60% of the activity of p365 in A9 cells (compare lanes 1 and 3), was almost completely abolished in the *JunB* expressor AJB1 cells (Fig. 3D, compare lanes 3 and 4). The construct p142 (Fig. 3B,3D), as well as p38 (not shown) were inactive in both cell lines.

The moderate repression of p365 activity in *JunB* A9 compared to the striking decrease of p190 activity
30 suggests that the region -190 to -142 includes the major target for *JunB* transrepression. This would probably be an indirect target, due to lack of known AP1 binding sites in this region. In addition the region -365 to -190 seems to include a site that interferes actively with the *JunB* suppression of the H-2 promoter. Possibly, the binding of the cJun-cFos complex to the AP1 site at -200 -193, is capable of preventing (partially) the repression by *JunB*. A possible mechanism may involve competitive interactions between
35 *JunB*, cJun and cFos.

MODULATION OF TUMORIGENIC AND METASTATIC POTENCY

Transfected clones were tested for their tumorigenic and metastatic properties in syngeneic C57BL/6
40 mice which are the original host strain of 3LL and B16 tumors.

Fig. 5A demonstrates the mean growth rates of local tumors from the parental D122 clone and from transfected cell lines. Mice were sacrificed at the time of death of control groups. Lung weights were evaluated as described in experimental procedures. Two independent experiments clearly indicated that the
45 *c-jun* transfectants grew at similar rates to the parental, highly tumorigenic D122 cells. On the other hand, in mice inoculated with the *c-fos* transfectant, F6A2, the growth of the tumor was significantly slower ($p < 0.005$) and in the groups that were inoculated with high expressor *c-fos+c-jun* transfectants (FJ23, D6, D13, D56) the tumors did not grow at all in 92% (45 out of 49) of the mice.

To test the ability of these D122 transfectants to metastasize spontaneously in C57BL/6 recipients, primary tumors were amputated when the tumor reached 8mm in diameter and formation of lung metastasis
50 was monitored in moribund mice.

Mice in the control, D122 injected, group, as well as the *c-jun* transfected groups showed very high levels of metastases 65 to 70 days after injection (data not shown). Mice injected with *c-fos* transfectants showed similar levels of metastases 85 to 90 days after injection. In contrast, among mice in six groups injected with double transfectants, 95% were metastasis free 120 days after injection indicating that a
55 complete eradication of tumor cells had occurred at the primary injection site.

Taken together, these spontaneous metastasis assays, which closely parallel the course of disease in human, suggest an absolute suppression of the parental tumorigenic and metastatic phenotype following *c-fos+c-jun* cotransfection. This was not due to intrinsic growth inhibitory effects on the cells, since in

vitro all transfectants proliferated at a rate similar to that of the parental cells (data not shown).

To test whether the nonmetastatic phenotype manifested by the *c-fos + c-jun* transfectants is due to their failure to grow in the foot pad or whether the reduction in metastatic potential will also be manifested by an experimental metastasis assay, these clones were injected intravenously to C57BL/6 mice. The mice were sacrificed when the mice in the control D122 group showed signs of respiratory distress, generally 35 to 39 days after injection. The lungs were excised and metastatic loads were evaluated.

Figures 5C and 6 A-B show the results of two i.v. experiments. The results are consistent with results of the spontaneous (intra footpad) experiments. Both the parental D122 and the *c-jun* transfectants were highly metastatic, the *c-fos* transfected group were of moderate metastatic potential and the double *c-fos + c-jun* transfectants were of low or nonmetastatic phenotype. Thus, low tumorigenicity and low metastatic potential were fully correlated for the D122 *c-fos + c-jun* transfectants. In randomly selected 3LL clones, no such correlation was previously observed between the high and low metastatic clones (6).

Consistent with the above results are also the experimental metastasis assays with the B16-F10.9, *c-fos + c-jun* transfected clones. As shown in Fig. 5C and 6C, the high expressors were of low metastatic phenotype, as opposed to the high metastatic phenotype of the parental clone.

IMMUNOGENICITY OF THE *c-fos*, *c-jun* TRANSFECTANTS

To test *in vivo* whether the low metastatic phenotype of the double transfectants is the result of an interaction with the host's T cell dependent immune system, tumorigenicity and metastasis assays were performed in CD1 nude mice which were deficient in thymic dependent mature T cells.

Fig. 5B shows the growth curves of *c-fos + c-jun* transfectants, of a *c-jun* transfectant, and of the parental D122 clone. The growth of the double transfectants is similar to the growth rates of the *c-jun* transfectant in the nude mice. Interestingly, all types of transfectants grew slightly slower than the D122 cells in nude mice. These clones exhibited a high metastatic phenotype, both in spontaneous and experimental assays (data not shown). Thus, the regain of the tumorigenic and metastatic properties by the *c-fos + c-jun* transfectants suggests that the suppression of the parental phenotype manifested in the C57BL mice is a result of an elevated immunogenicity which is dependent on a mature T-cell reaction.

Further evidence to the acquired immunogenicity was observed when mice that had been injected by the double transfectants and did not show growth of the primary tumor after 120-150 days (Fig. 5A) were reinjected, intrafootpad, with the parental, high metastatic, D122 cells. In groups previously injected by D13 or FJ23 cells, the D122 cells grew significantly slower than in the control group ($p < 0.005$ compared to naive mice injected with D122 cells), while groups previously injected by D6 or D56 cells showed a smaller, less significant reduction of tumor growth (data not shown). These results suggest that the rejection of the primary tumors created memory cells capable of interacting with a D122 challenge.

The correlation between CTL activity and the malignant phenotypes of the various transfectants were tested. Figure 7 shows the autologous, *in vitro* lytic activity of CTLs elicited in C57BL mice by inactivated (irradiated) D122 clone, *c-fos* transfectant F6A2, *c-jun* transfectant J67 and *c-fos + c-jun* cotransfectant clone D13. *In vitro* resensitized splenocytes were reacted with homologous S³⁵-methionine labeled target cells in 16 hours assays. Spontaneous release was under 20% of total release. Triplicate samples were under 5% mean error. Immunization of D122 and J67 elicits low levels of CTL against autologous D122 or J67. In contrast, immunization by the *c-fos* transfectants or by *c-fos + c-jun* cotransfectants, elicit higher levels of CTL, that lyse efficiently autologous targets. These data are in agreement with *in vivo* results, and indicate that CTL induction and sensitivity might be a major effector in inhibition of tumorigenicity and metastasis in these transfectants.

JunB TRANSFECTION CONVERTS LOW TO HIGH METASTATIC CELLS

The negative effect of *junB* transfection of MHC class I expression of A9 (3LL) cells raised the question of the potential effect of *junB* on the metastatic potential. Thus, the *junB* A9 transfected clones (AJB1 and AJB10) were tested for their tumorigenic and metastatic properties in syngeneic C57BL/6 mice. Spontaneous metastasis assays indicated that the local tumor in mice inoculated by the transfectants did not grow significantly faster than the parental A9 clone and no spontaneous metastases developed up to 150 days after amputation. Conversely, when injected directly into the vein, six out of seven mice from the AJB1 group developed metastases 65 days post injection. Thereafter all other mice were sacrificed. Three out of seven mice from the AJB10 group but none of the A9 parental group developed metastases before the cessation of the experiment. Figures 5C (right hand), and Figure 6D demonstrate the i.v. results; while parental A9 cells were nonmetastatic, *junB* transfectants were moderately (AJB10) or high (AJB1) meta-

static. Based on the previous result, the increase in the metastatic potency following *junB* transfection is a result of reduced immunogenicity and reduced susceptibility to T-cell lysis.

The above data provide clear evidence that *c-fos*, *c-jun* and *junB* over-expression suppress certain aspects of the cancer process in murine carcinoma and melanoma cells while transfection with *junB* induces a metastatic phenotype. Tumor progression and metastases is a highly complicated process dependent on positive and negative regulation of many genes. Expression of MHC class I genes, that is obligatory for production of cellular immunity, was shown to be a rate limiting step in metastasis of 3LL carcinoma (6), B16 melanoma, T10 sarcoma and a variety of other tumors (39). The data above show a link between expression of MHC class I and expression of *c-fos* and *jun* family genes.

The above results further show that vaccines of human tumors can be generated by transfer of MHC genes and particularly, with two parental MHC genes tailor made for the heterozygous HLA phenotype of each patient. A simpler approach is to activate the expression of the endogenous MHC genes of each heterozygous tumor. This was achieved in accordance with the above experimental data by transfecting tumor cells with the *c-fos* and *c-jun* genes or the *c-fos* gene alone. This is based on conclusions drawn from the data wherein the nonmetastatic H-2K expression clones of the 3LL tumor coexpress the *c-fos* gene. Accordingly, the question is raised whether the expression of the two gene products is causally related and whether the *c-fos* gene is involved in the up regulation of MHC class I genes. Earlier studies (7,8) by applicants indicated that the application in culture of γ -Interferon to D122 cells induced the expression of H-2 genes. The above data demonstrates that following treatment with Interferon, first *c-fos* transcripts were induced, followed by the appearance of H-2K transcripts. Transfection with *c-fos* gene, whether of mouse (SEQ ID No:5) or of human (SEQ ID No:6) origin, converted the H-2K nonexpressors to expressor cells. When such cells were transplanted to syngeneic recipients, the generation of metastasis was significantly reduced.

Other systems in which the *c-fos* was shown to control the expression of cellular genes indicated that the *c-fos* protein does not bind by itself to promote a region, but rather forms through a leucine zipper heterodimers of *c-fos* and *c-jun*, *junB*, or *junD*. These heterodimers act as the nuclear effectors by binding to DNA consensus sequences. The data shows that transfection of tumor cells with *c-fos* induced *c-jun* and *junB* expression in reciprocally transfection with *c-jun* or *c-fos* expression. However, the *c-fos* transfectants manifested a reduction, but not complete abolishment, of the metastatic phenotype. Cotransfection of D122 cells with *c-fos* and *c-jun* genes resulted in a significant coexpression of both genes and up-regulation of H-2 genes. When such double transfectants were then transplanted into syngeneic animals, they manifested a complete abolishment of their phenotype. In fact, even the local growth of the transfectants was significantly arrested.

It appears that the insertion of both the *c-fos* and *c-jun* genes resulted in the acquisition of a very effective immunogenic potency. The increase in immunogenicity of double transfectants was not attributed just to the up-regulation of the H-2K expression, since the levels of the H-2K transcripts following *c-fos* transcription was similar to the levels induced following *c-fos* or *c-jun* gene transfer. It is possible that in the double transfectants, only the class I gene products were elevated. The expression of tumorous associated antigens or their process to cell surface peptides could also have been up-regulated following transfection with the *c-fos* and *c-jun* genes. The complete abolishment of the metastatic phenotype due to the acquisition of potent immunogenic properties following transfection with the *c-fos* and *c-jun* gene was achieved also with the B16-F10.9 melanoma. Accordingly, the insertion of the *c-fos* and *c-jun* genes which control the expression of MHC class I genes is a useful strategy for the generation of cellular tumor vaccines made in accordance with the methods described above, avoiding the necessity to tailor-make gene insertion in accordance with the individual MHC phenotypes.

Studies were conducted on a number of lines of human tumor cells. Applying differentiation inducers to such cells in culture, it was observed that whenever terminal differentiation was involved in the up-regulation of HLA expression, *c-fos* transcripts proceeded the appearance of the HLA transcripts. No *c-fos* expression was observed when the differentiation pattern did not involve HLA expression. It is therefore believed that transfer of *c-fos* and *c-jun* genes into human neoplastic cells aiming at increased immunogenicity, is a modality for the generation of cellular vaccines of human tumors. Immunotherapy via gene therapy then comprises the surgical removal of the primary tumor, followed by vaccination with inactivated tumor cells into which *c-fos* and *c-jun* or *c-jun* alone had been inserted, resulting in highly immunogenic vaccine in accordance with the present invention.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims the invention may be practiced otherwise than as specifically described.

5 REFERENCES

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Eisenbach, Lea
Feldman, Michael

(ii) TITLE OF INVENTION: Anti-Metastatic Vaccine

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Reising, Ethington, Barnard, Perry & Milton
(B) STREET: P. O. Box 4390
(C) CITY: Troy
(D) STATE: Michigan
(E) COUNTRY: U.S.A.
(F) ZIP: 48099

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/968,415
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Kohn, Kenneth I.
(B) REGISTRATION NUMBER: 30,955
(C) REFERENCE/DOCKET NUMBER: P-301(Weiz 28-92)

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (313) 689-3500
(B) TELEFAX: (313) 689-4071

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3135 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: mRNA
(B) LOCATION: 1..3135

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 917..1921

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	TCTCACCAAC TGCTTGGATC CAGCGCCCGC GGCTCCTGCA CCGGTATTTT GGGGAGCATT	180
	TGGAGAGTCC CTTCTCCCGC CTTCCACGGA GAAGAAGCTC ACAAGTCCGG GCGCTGCTGA	240
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	CTTTCCTGAC CCAGAGGACC GGTAACAAGT GGCCGGGAGC GAACTTTTGC AAATCTCTTC	360
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	ACCCTGCGCT CCCTGGAGCG AACTGGGGAG GAGGGCTCAG GGGGAAGCAC TGCCGTCTGG	780
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30	GAT GCC CTC AAC GCC TCG TTC CTC CAG TCC GAG AGC GGT GCC TAC GGC	997
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	80 85 90	
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EP 0 599 077 A2

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	GAG GAA AAA GTG AAA ACC TTG AAA GCG CAA AAC TCC GAG CTG GCA TCC	1813
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 30 (A) LENGTH: 334 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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 20 25 30
 40 Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala Asp Pro Val Gly Ser
 35 40 45
 Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp Leu Leu Thr Ser Pro
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 65 70 75 80
 Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr Pro Thr Pro Thr Gln
 85 90 95
 50 Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln Glu Gly Phe Ala Glu
 100 105 110

55

EP 0 599 077 A2

Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser Gln Asn Thr Leu Pro
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 180 185 190
 Ala Gly Leu Ala Phe Pro Ser Gln Pro Gln Gln Gln Gln Gln Pro Pro
 195 200 205
 Gln Pro Pro His His Leu Pro Gln Gln Ile Pro Val Gln His Pro Arg
 210 215 220
 Leu Gln Ala Leu Lys Glu Glu Pro Gln Thr Val Pro Glu Met Pro Gly
 225 230 235 240
 Glu Thr Pro Pro Leu Ser Pro Ile Asp Met Glu Ser Gln Glu Arg Ile
 245 250 255
 Lys Ala Glu Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys
 260 265 270
 Arg Lys Arg Lys Leu Glu Arg Ile Ala Arg Leu Glu Glu Lys Val Lys
 275 280 285
 Thr Leu Lys Ala Gln Asn Ser Glu Leu Ala Ser Thr Ala Asn Met Leu
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 Arg Glu Gln Val Ala Gln Leu Lys Gln Lys Val Met Asn His Val Asn
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 Ser Gly Cys Gln Leu Met Leu Thr Gln Gln Leu Gln Thr Phe
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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3622 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA
 (ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION: 287..3622
 (ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION: 289..3622

(ix) FEATURE:

(A) NAME/KEY: mRNA

(B) LOCATION: 293..3622

5

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1261..2256

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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 GAGCTGGTGA GGAGGGCGCA GCGGGGACGA CAGCCAGCGG GTGCGTGCGC TCTTAGAGAA 1140
 40 ACTTTCCCTG TCAAAGGCTC CGGGGGGCGC GGGTGTCGCC CGCTTGCCAG AGCCCTGTTG 1200
 CGGCCCCGAA ACTTGTGCGC GCACGCCAAA CTAACCTCAC GTGAAGTGAC GGAAGTTTCT 1260
 ATG ACT GCA AAG ATG GAA ACG ACC TTC TAT GAC GAT GCC CTC AAC GCC 1308
 Met Thr Ala Lys Met Glu Thr Thr Phe Tyr Asp Asp Ala Leu Asn Ala
 1 5 10 15
 45 TCG TTC CTC CCG TCC GAG AGC GGA CCT TAT GGC TAC AGT AAC CCC AAG 1356
 Ser Phe Leu Pro Ser Glu Ser Gly Pro Tyr Gly Tyr Ser Asn Pro Lys
 20 25 30
 50 ATC CTG AAA CAG AGC ATG ACC CTG AAC CTG GCC GAC CCA GTG GGG AGC 1404
 Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala Asp Pro Val Gly Ser
 35 40 45

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EP 0 599 077 A2

	CTG AAG CCG CAC CTC CGC GCC AAG AAC TCG GAC CTC CTC ACC TCG GCC	1452
	Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp Leu Leu Thr Ser Pro	
	50 55 60	
5	GAC GTG GGG CTG CTC AAG CTG GCG TCG CCC GAG CTG GAG CGC CTG ATA	1500
	Asp Val Gly Leu Leu Lys Leu Ala Ser Pro Glu Leu Glu Arg Leu Ile	
	65 70 75 80	
	ATC CAG TCC AGC AAC GGG CAC ATC ACC ACC ACG CCG ACC CCC ACC CAG	1548
	Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr Pro Thr Pro Thr Gln	
10	85 90 95	
	TTC CTG TGC CCC AAG AAC GTG ACA GAT GAG CAG GAG GGG TTC GCC GAG	1596
	Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln Glu Gly Phe Ala Glu	
	100 105 110	
15	GGC TTC GTG CCG GCC CTG GCC GAA CTG CAC AGC CAG AAC ACG CTG CCC	1644
	Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser Gln Asn Thr Leu Pro	
	115 120 125	
	AGC GTC ACG TCG GCG GCG CAG CCG GTC AAC GGG GCA GGC ATG GTG GCT	1692
	Ser Val Thr Ser Ala Ala Gln Pro Val Asn Gly Ala Gly Met Val Ala	
20	130 135 140	
	CCC GCG GTA GCC TCG GTG GCA GGG GGC AGC GGC AGC GGC GGC TTC AGC	1740
	Pro Ala Val Ala Ser Val Ala Gly Gly Ser Gly Ser Gly Gly Phe Ser	
	145 150 155 160	
	GCC AGC CTG CAC AGC GAG CCG CCG GTC TAC GCA AAC CTC AGC AAC TTC	1788
	Ala Ser Leu His Ser Glu Pro Pro Val Tyr Ala Asn Leu Ser Asn Phe	
25	165 170 175	
	AAC CCA GGC GCG CTG AGC AGC GGC GGC GGG GCG CCC TCC TAC GGC GCG	1836
	Asn Pro Gly Ala Leu Ser Ser Gly Gly Gly Ala Pro Ser Tyr Gly Ala	
	180 185 190	
30	GCC GGC CTG GCC TTT CCC GCG CAA CCC CAG CAG CAG CAG CAG CCG CCG	1884
	Ala Gly Leu Ala Phe Pro Ala Gln Pro Gln Gln Gln Gln Gln Pro Pro	
	195 200 205	
	CAC CAC CTG CCC CAG CAG ATG CCC GTG CAG CAC CCG CCG CTG CAG GCC	1932
	His His Leu Pro Gln Gln Met Pro Val Gln His Pro Arg Leu Gln Ala	
35	210 215 220	
	CTG AAG GAG GAG CCT CAG ACA GTG CCC GAG ATG CCC GGC GAG ACA CCG	1980
	Leu Lys Glu Glu Pro Gln Thr Val Pro Glu Met Pro Gly Glu Thr Pro	
	225 230 235 240	
	CCC CTG TCC CCC ATC GAC ATG GAG TCC CAG GAG CCG ATC AAG GCG GAG	2028
	Pro Leu Ser Pro Ile Asp Met Glu Ser Gln Glu Arg Ile Lys Ala Glu	
40	245 250 255	
	AGG AAG CCG ATG AGG AAC CCG ATC GCT GCC TCC AAG TGC CGA AAA AGG	2076
	Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys Arg Lys Arg	
	260 265 270	
45	AAG CTG GAG AGA ATC GCC CCG CTG GAG GAA AAA GTG AAA ACC TTG AAA	2124
	Lys Leu Glu Arg Ile Ala Arg Leu Glu Glu Lys Val Lys Thr Leu Lys	
	275 280 285	
	GCT CAG AAC TCG GAG CTG GCG TCC ACG GCC AAC ATG CTC AGG GAA CAG	2172
	Ala Gln Asn Ser Glu Leu Ala Ser Thr Ala Asn Met Leu Arg Glu Gln	
50	290 295 300	

	GTG GCA CAG CTT AAA CAG AAA GTC ATG AAC CAC GTT AAC AGT GGG TGC	2220
	Val Ala Gln Leu Lys Gln Lys Val Met Asn His Val Asn Ser Gly Cys	
	305 310 315 320	
5	CAA CTC ATG CTA ACG CAG CAG TTG CAA ACA TTT TGAAGAGAGA CCGTCGGGGG	2273
	Gln Leu Met Leu Thr Gln Gln Leu Gln Thr Phe	
	325 330	
	CTCAGGGGCA ACCAAGAAAA AAAATAACAC AGAGAGACAG ACTTGAGAAC TTGACAAGTT	2333
10	GCGACGGAGA GAAAAAGAA GTGTCCGAGA ACTAAAGCCA AGGGTATCCA AGTTGGACTG	2393
	GGTTCGGTCT GACGGCGCCC CCAGTGTGCA CGAGTGGGAA GGACTTGGTC GCGCCCTCCC	2453
	TTGGCGTGGA GCCAGGGAGC GGCCCGCTGC GGGCTGCCCC GCTTTGCGGA CGGGCTGTCC	2513
15	CCGCGCGAAC GGAACGTTGG ACTTTCGTTA ACATTGACCA AGAACTGCAT GGACCTAACA	2573
	TTCGATCTCA TTCAGTATTA AAGGGGGGAG GGGGAGGGGG TTACAACTG CAATAGAGAC	2633
	TGTAGATTGC TTCTGTAGTA CTCCTTAAGA ACACAAAGCG GGGGGAGGGT TGGGGAGGGG	2693
	CGGCAGGAGG GAGGTTTGTG AGAGCGAGGC TGAGCCTACA GATGAACTCT TTCTGGCCTG	2753
20	CTTTCGTTAA CTGTGTATGT ACATATATAT ATTTTAAAT TTGATTAAAG CTGATTACTG	2813
	TCAATAACA GCTTCATGCC TTTGTAAGTT ATTTCTTGT TGTITGTTT GGTATCCTGC	2873
	CCAGTGTGTG TTGTAAATAA GAGATTGGA GCACTCTGAG TTTACCATTG GTAATAAAGT	2933
25	ATATAATTTT TTTATGTTTT GTTCTGAAA ATTCCAGAAA GGATATTTAA GAAAATACAA	2993
	TAAACTATTG GAAAGTACTC CCCTAACCTC TTTTCTGCAT CATCTGTAGA TCCTAGTCTA	3053
	TCTAGGTGGA GTTGAAAGAG TTAAGAATGC TCGATAAAAT CACTCTCAGT GCTTCTTACT	3113
30	ATTAAGCAGT AAAAAGTGT CTCTATTAGA CTTAGAAATA AATGTACCTG ATGTACCTGA	3173
	TGCTATGTCA GGCTTCATAC TCCACGCTCC CCCAGCGTAT CTATATGGAA TTGCTTACCA	3233
	AAGGCTAGTG CGATGTTTCA GGAGGCTGGA GGAAGGGGGG TTGCAGTGA GAGGGACAGC	3293
	CCACTGAGAA GTCAAACATT TCAAAGTTG GATTGCATCA AGTGGCATGT GCTGTGACCA	3353
35	TTTATAATGT TAGAAATTTT ACAATAGGTG CTTATTCTCA AAGCAGGAAT TGGTGGCAGA	3413
	TTTTACAAA GATGTATCCT TCCAATTGG AATCTTCTCT TTGACAATTC CTAGATAAAA	3473
	AGATGGCCTT TGTCTTATGA ATATTTATAA CAGCATTCTG TCACAATAAA TGTATTCAAA	3533
40	TACCAATAAC AGATCTTGAA TTGCTTCCCT TTACTACTTT TTTGTCCCA AGTTATATAC	3593
	TGAAGTTTTT ATTTTAGTT GCTGAGGTT	3622

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 331 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Ala Lys Met Glu Thr Thr Phe Tyr Asp Asp Ala Leu Asn Ala
 1 5 10 15
 Ser Phe Leu Pro Ser Glu Ser Gly Pro Tyr Gly Tyr Ser Asn Pro Lys
 20 25 30
 Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala Asp Pro Val Gly Ser
 35 40 45
 Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp Leu Leu Thr Ser Pro
 50 55 60
 Asp Val Gly Leu Leu Lys Leu Ala Ser Pro Glu Leu Glu Arg Leu Ile
 65 70 75 80
 Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr Pro Thr Pro Thr Gln
 85 90 95
 Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln Glu Gly Phe Ala Glu
 100 105 110
 Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser Gln Asn Thr Leu Pro
 115 120 125
 Ser Val Thr Ser Ala Ala Gln Pro Val Asn Gly Ala Gly Met Val Ala
 130 135 140
 Pro Ala Val Ala Ser Val Ala Gly Gly Ser Gly Ser Gly Gly Phe Ser
 145 150 155 160
 Ala Ser Leu His Ser Glu Pro Pro Val Tyr Ala Asn Leu Ser Asn Phe
 165 170 175
 Asn Pro Gly Ala Leu Ser Ser Gly Gly Gly Ala Pro Ser Tyr Gly Ala
 180 185 190
 Ala Gly Leu Ala Phe Pro Ala Gln Pro Gln Gln Gln Gln Pro Pro
 195 200 205
 His His Leu Pro Gln Gln Met Pro Val Gln His Pro Arg Leu Gln Ala
 210 215 220
 Leu Lys Glu Glu Pro Gln Thr Val Pro Glu Met Pro Gly Glu Thr Pro
 225 230 235 240
 Pro Leu Ser Pro Ile Asp Met Glu Ser Gln Glu Arg Ile Lys Ala Glu
 245 250 255
 Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys Arg Lys Arg
 260 265 270
 Lys Leu Glu Arg Ile Ala Arg Leu Glu Glu Lys Val Lys Thr Leu Lys
 275 280 285
 Ala Gln Asn Ser Glu Leu Ala Ser Thr Ala Asn Met Leu Arg Glu Gln
 290 295 300
 Val Ala Gln Leu Lys Gln Lys Val Met Asn His Val Asn Ser Gly Cys
 305 310 315 320
 Gln Leu Met Leu Thr Gln Gln Leu Gln Thr Phe
 325 330

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3548 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:
 (A) NAME/KEY: TATA_signal
 (B) LOCATION: 101..106

(ix) FEATURE:
 (A) NAME/KEY: polyA_signal
 (B) LOCATION: 3493..3498

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 284..424

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 1179..1430

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 1836..1943

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2061..2702

(ix) FEATURE:
 (A) NAME/KEY: precursor_RNA
 (B) LOCATION: 133..2702

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 425..1178

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 1431..1835

(ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 1944..2060

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAGTTGACGA CAGAGCGCCC GCAGAGGGCC TTGGGGCGCG CTTCCCCCCC CTTCCAGTTC	60
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CGCCTACTAC TCCAACCGCG ACTGCAGCGA GCAACTGAGA AGACTGGATA GAGCCGGCGG	180
TTCCGCGAAC GAGCAGTGAC CGCGCTCCCA CCCAGCTCTG CTCTGCAGCT CCCACCACTG	240
TCTACCCCTG GACCCCTTGC CGGGCTTTCC CCAACTTCG ACCATGATGT TCTCGGGTTT	300
CAACGCCGAC TACGAGGCGT CATCTCCCG CTGCAGTAGC GCCTCCCCGG CCGGGGACAG	360
CCTTTCCTAC TACCATTCCC CAGCCGACTC CTTCTCCAGC ATGGGCTCTC CTGTCAACAC	420

EP 0 599 077 A2

	ACAGGTGAGT TTGGCTTTGT GTAGCCGCCA GGTCCGCGCT GAGGGTCGCC GTGGAGGAGA	480
	CACTGGGGTG TGA CTCCAG GGGCGGGGGG GTCTTCCTTT TTCGCTCTGG AGGGAGACTG	540
5	GCGCGGTCAG AGCAGCCTTA GCCTGGGAAC CCAGGACTTG TCTGAGCGCG TGCACACTTG	600
	TCATAGTAAG ACTTAGTGAC CCCTTCCCGC GCGGCAGGTT TATTCTGAGT GGCCTGCCTG	660
	CATTCTTCTC TCGGCCGACT TGTTCCTGAG ATCAGCCGGG GCCAACAAGT CTCGAGCAAA	720
10	GAGTCGCTAA CTAGAGTTTG GGAGCGGCA AACCCCGCA ATCCCCCTC CCGGGGCAGC	780
	CTGGAGCAGG GAGGAGGGAG GAGGGAGGAG GGTGCTGCGG GCGGGTGTGT AAGGCAGTTT	840
	CATTGATAAA AAGCGAGTTC ATTCTGGAGA CTCCGGAGCA GCGCCTGCGT CAGCGCAGAC	900
15	GTGAGGGATA TTTATAACAA ACCCCCTTTC GAGCGAGTGA TGCCGAAGGG ATAACGGGAA	960
	CGCAGCAGTA GGATGGAGGA GAAAGGCTGC GCTGCGGAAT TCAAGGGAGG ATATTGGGAG	1020
	AGCTTTTATC TCCGATGAGG TGCATACAGG AAGACATAAG CAGTCTCTGA CCGGAATGCT	1080
	TCTCTCTCCC TGCTTCATGC GACACTAGGG CCAGTTGCTC CACCTGTGTC TGGAACCTCC	1140
20	TCGCTCACCT CCGCTTTCCT CTTTTGTTT TGTTCAGGA CTTTTCGCA GATCTGTCCG	1200
	TCTCTAGTGC CAACTTTATC CCCACGGTGA CAGCCATCTC CACCAGCCCA GACCTGCAGT	1260
	GGCTGGTGCA GCGCACTCTG GTCTCCTCCG TGGCCCCATC GCAGACCAGA GCGCCCCATC	1320
25	CTTACGGACT CCCCACCCAG TCTGCTGGGG CTTACGCCAG AGCGGGAATG GTGAAGACCG	1380
	TGTCAGGAGG CAGAGCGCAG AGCATCGGCA GAAGGGGCAA AGTAGAGCAG GTGAGCAGCG	1440
	ATTCTGGACC TTTGTGGGCT GGGGGGGGGG GGGGGGGCGG AGACTGACGC ACAGACCACA	1500
30	CAACAGAGAA GGGACGCTAC TGA CTGCACT TCCTGACCAG GAGCTGTGGC TGCTAGCCCT	1560
	TTCCCTCCCT TGTCAGATTT TGACAGTTGG ACCCAAGACA AACTCTAGAC AGTTTCCCTG	1620
	ACAGCTTCCT ACTTCATTCT CTAGCCGGGG AGCTTCTTTG TTCCCTGCT AAAGATCTCA	1680
	CTTTAAATGC AAATCACACT CTGCCTGCCA ACTGCAGGTT AGAAAACTG CTTACCCGAG	1740
35	AGGTGCGGGT GCTGTAGGAG CCAGTTTAC TGGGGTCACT GAATGGAGGT GACACTAGAC	1800
	AACCTTAACT GAATGTTGGT CCTTTCTTC TATAGCTATC TCCTGAAGAG GAAGAGAAAC	1860
	GGAGAATCCG AAGGGAACGG AATAAGATGG CTGCAGCCAA GTGCCGGAAT CGGAGGAGGG	1920
40	AGCTGACAGA TACACTCCAA GCGGTAGGTT GAACCAAGCTG CTGCTCCTGA AACTTTATTA	1980
	AAGTTGGAGC TTGGGACTAT GGGCGCAGGG TCCTTGAGCA TGCCCGTGTC TTATGCTTTC	2040
	TTATATCTCT CCCTATGCAG GAGACAGATC AACTTGAAGA TGAGAAGTCT GCGTTGCAGA	2100
45	CTGAGATTGC CAATCTGCTG AAAGAGAAGG AAAAACTGGA GTTTATTTTG GCAGCCCACC	2160
	GACCTGCCTG CAAGATCCCC GATGACCTTG GCTTCCGAGA GGAGATGTCT GTGGCCTCCC	2220
	TGGATTTGAC TGGAGGTCTG CCTGAGGCTT CCACCCGAGA GTCTGAGGAG GCCTTCACCC	2280
	TGCCCCTTCT CAACGACCCT GAGCCCCAAGC CATCCTTGA GGCAGTCAAG AGCATCAGCA	2340
50	ACGTGGAGCT GAAGGCAGAA CCCTTTGATG ACTTCTTGT TCCGGCATCA TCTAGGCCCA	2400
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EP 0 599 077 A2

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5  GTGGCTCAGA GACCTCCCGC TCTGTGCCAG ATGTGGACCT GTCCGGTTCC TTCTATGCAG 2460
   CAGACTGGGA GCCTCTGCAC AGCAATTCCT TGGGGATGGG GCCCATGGTC ACAGAGCTGG 2520
   AGCCCCGTGT TACTCCCGTG GTCACCTGTA CTCCGGGCTG CACTACTTAC ACGTCTTCCT 2580
   TTGTCTTCAC CTACCCTGAA GCTGACTCCT TCCCAAGCTG TGCCGCTGCC CACCGAAAGG 2640
   GCAGCAGCAG CAACGAGCCC TCCTCCGACT CCCTGAGCTC ACCCACGCTG CTGGCCCTGT 2700
10  GAGCAGTCAG AGAAGGCAAG GCAGCCGGCA TCCAGACGTG CCACTGCCCC AGCTGGTGCA 2760
   TTACAGAGAG GAGAAACACG TCTTCCCTCG AAGGTTCCCG TCGACCTAGG GAGGACCTTA 2820
   CCTGTTCGTG AAACACACCA GGCTGTGGGC CTCAAGGACT TGCAAGCATC CACATCTGGC 2880
   CTCCAGTCCT CACCTCTTCC AGAGATGTAG CAAAAACAAA ACAAACAAA ACAAAAAACC 2940
15  GCATGGAGTG TGTGTTCTCT AGTGACACCT GAGAGCTGGT AGTTAGTAGA GCATGTGAGT 3000
   CAAGGCCTGG TCTGTGTCTC TTTTCTCTTT CTCCTTAGTT TTCTCATAGC ACTAACTAAT 3060
   CTGTTGGGTT CATTATTGGA ATTAACCTGG TGCTGGATTG TATCTAGTGC AGCTGATTTT 3120
20  AACAAACCT ACTGTGTTCC TGGCAATAGC GTGTTCCAAT TAGAAACGAC CAATATTAAA 3180
   CTAAGAAAAG ATAGGACTTT ATTTTCCAGT AGATAGAAAT CAATAGCTAT ATCCATGTAC 3240
   TGTAGTCCTT CAGCGTCAAT GTTCATTGTC ATGTTACTGA TCATGCATTG TCGAGGTGGT 3300
25  CTGAATGTTT TGACATTAACT AGTTTCCCAT GAAAACGTTT TTATTGTGTT TTCAATTTAT 3360
   TTATTAAGAT GGATTCTCAG ATATTTATAT TTTTATTTTA TTTTTTCTA CCCTGAGGTC 3420
   TTTCGACATG TGGAAAGTGA ATTTGAATGA AAAATTTTAA GCATTGTTTG CTTATTGTTT 3480
30  CAGGACATTG TCAATAAAG CATTAAAGT GAATGCGACC ACCTTCTTGC TCTCTTTATT 3540
   CTCAGTTT 3548

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(2) INFORMATION FOR SEQ ID NO:6:

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   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 6210 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
40  (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: DNA (genomic)

   (ix) FEATURE:
45  (A) NAME/KEY: CDS
       (B) LOCATION: join(889..1029, 1783..2034, 2466..2573, 2688
           ..3329)

   (ix) FEATURE:
       (A) NAME/KEY: misc feature
50  (B) LOCATION: 402..453
       (D) OTHER INFORMATION: /note= "transcriptional activator
           region"

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(ix) FEATURE:
 (A) NAME/KEY: prim transcript
 (B) LOCATION: 734..3329

5 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 889..1029
 (D) OTHER INFORMATION: /note= "c-fos protein, exon1"

10 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 1030..1782
 (D) OTHER INFORMATION: /note= "c-fos, intron A"

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 1783..2034

15 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2035..2465

(ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2466..2573

20 (ix) FEATURE:
 (A) NAME/KEY: intron
 (B) LOCATION: 2574..2687

25 (ix) FEATURE:
 (A) NAME/KEY: exon
 (B) LOCATION: 2688..3329

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

30 GCAGGAACAG TGCTAGTATT GCTCGAGCCC GAGGGCTGGA GGTTAGGGGA TGAAGGTCTG 60
 CTTCACGCT TTGCACTGAA TTAGGGCTAG AATTGGGGAT GGGGGTAGGG GCGCATTCTT 120
 TCGGGAGCCG AGGCTTAAAGT CCTCGGGGTC CTGTACTCGA TGCCGTTTCT CCTATCTCTG 180
 35 AGCCTCAGAA CTGTCTTCAG TTTCCGTACA AGGGTAAAAA GGCGCTCTCT GCCCCATCCC 240
 CCCCACCTC GGAACAAGG GTCCGCATTG AACCAGGTGC GAATGTTCTC TCTCATTCTG 300
 CGCCGTTCCG GCCTCCCTC CCCCAGCCGC GGCCCCCGCC TCCCCCGCA CTGCACCCTC 360
 40 GGTGTTGGCT GCAGCCCGCG AGCAGTTCCT GTCAATCCCT CCCCCTTAC ACAGGATGTC 420
 CATATTAGGA CATCTGCGTC AGCAGGTTTC CACGGCCTTT CCCTGTAGCC CTGGGGGGAG 480
 CCATCCCCGA AACCCTCAT CTTGGGGGGC CCACGAGACC TCTGAGACAG GAACTGCCAA 540
 45 ATGCTCACGA GATTAGGACA CGCGCCAAGG CGGGGGCAGG GAGCTCCGAG CGCTGGGGAC 600
 GCAGCCGGGC GGCCGCAGAA GCGCCCAGGC CCGCGGCCA CCCCTCTGGC GCCACCGTGG 660
 TTAGCCCGT GACGTTTACA CTCATTATA AAACGCTTGT TATAAAGCA GTGGCTGCGG 720
 CGCCTCGTAC TCCAACCGCA TCTGCAGCGA GCAACTGAGA AGCCAAGACT GAGCCGGCGG 780
 50 CCGCGGCGCA GCGAACGAGC AGTGACCGTG CTCCTACCCA GCTCTGCTTC ACAGCGCCCA 840

EP 0 599 077 A2

	CCTGTCTCCG CCCCTCGGCC CCTCGCCCGG CTTTGCCTAA CCGCCACG ATG ATG TTC Met Met Phe 1	897
5	TCG GGC TTC AAC GCA GAC TAC GAG GCG TCA TCC TCC CGC TGC AGC AGC Ser Gly Phe Asn Ala Asp Tyr Glu Ala Ser Ser Ser Arg Cys Ser Ser 5 10 15	945
	GCG TCC CCG GCC GGG GAT AGC CTC TCT TAC TAC CAC TCA CCC GCA GAC Ala Ser Pro Ala Gly Asp Ser Leu Ser Tyr Tyr His Ser Pro Ala Asp 20 25 30 35	993
10	TCC TTC TCC AGC ATG GGC TCG CCT GTC AAC GCG CAG GTAAGGCTGG Ser Phe Ser Ser Met Gly Ser Pro Val Asn Ala Gln 40 45	1039
	CTTCCCGTCG CCGCGGGGCC GGGGGCTTGG GGTCCGGGAG GAGGAGACAC CGGGCGGGAC	1099
15	GCTCCAGTAG ATGAGTAGGG GGCTCCCTTG TGCCTGGAGG GAGGCTGCCG TGGCCGGAGC	1159
	GGTCCCGGCT CGGGGGCTCG GGACTTGCTC TGAGCGCACG CACGCTTGCC ATAGTAAGAA	1219
	TTGGTTCCCC CTTCGGGAGG CAGGTTCGTT CTGAGCAACC TCTGGTCTGC ACTCCAGGAC	1279
20	GGATCTCTGA CATTAGCTGG AGCAGACGTG TCCCAAGCAC AAACCTCGCTA ACTAGAGCCT	1339
	GGCTTCTTCG GGGAGGTGGC AGAAAGCGGC AATCCCCCCT CCCCCGGCAG CCTGGAGCAC	1399
	GGAGGAGGGA TGAGGGAGGA GCGTGCAGCG GCGGGCTGTG TAAGGCAGTT TCATTGATAA	1459
25	AAAGCGAGTT CATTCTGGAG ACTCCGGAGC GCGCGCTGCG TCAGCGCAGA CGTCAGGGAT	1519
	ATTATAACA AACCCCTTT CAAGCAAGTG ATGCTGAAGG GATAACGGGA ACGCAGCGGC	1579
	AGGATGGAAG AGACAGGCAC TGCGCTGCGG AATGCCTGGG AGGAAAAGGG GGAGACCTTT	1639
	CATCCAGGAT GAGGGACATT TAAGATGAAA TGTCCGTGGC AGGATCGTTT CTCTTCACTG	1699
30	CTGCATGCGG CACTGGGAAC TCGCCCCACC TGTGTCCGGA ACCTGCTCGC TCACGTCCGC	1759
	TTTCCCTTC TGTTTGTTC TAG GAC TTC TGC ACG GAC CTG GCC GTC TCC Asp Phe Cys Thr Asp Leu Ala Val Ser 50 55	1809
35	AGT GCC AAC TTC ATT CCC ACG GTC ACT GCC ATC TCG ACC AGT CCG GAC Ser Ala Asn Phe Ile Pro Thr Val Thr Ala Ile Ser Thr Ser Pro Asp 60 65 70	1857
	CTG CAG TGG CTG GTG CAG CCC GCC CTC GTC TCC TCT GTG GCC CCA TCG Leu Gln Trp Leu Val Gln Pro Ala Leu Val Ser Ser Val Ala Pro Ser 75 80 85	1905
40	CAG ACC AGA GCC CCT CAC CCT TTC GGA GTC CCC GCC CCC TCC GCT GGG Gln Thr Arg Ala Pro His Pro Phe Gly Val Pro Ala Pro Ser Ala Gly 90 95 100	1953
45	GCT TAC TCC AGG GCT GGC GTT GTG AAG ACC ATG ACA GGA GGC CGA GCG Ala Tyr Ser Arg Ala Gly Val Val Lys Thr Met Thr Gly Gly Arg Ala 105 110 115 120	2001
	CAG AGC ATT GGC AGG AGG GGC AAG GTG GAA CAG GTGAGGAAC CTAGCGTACT Gln Ser Ile Gly Arg Arg Gly Lys Val Glu Gln 125 130	2054
50	CTTCCTCGGA ATGTGGGGGC TGGGTGGGAA GCAGCCCCGG AGATGCAGGA GCCCAGTACA	2114

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	GAGGATGAAG CCACTGATGG GGCTGGCTGC ACATCCGTAA CTGGGAGCCC TGGCTCCAAG	2174
	CCCATTCCAT CCCAACTCAG ACTCTGAGTC TCACCCTAAG AAGTACTCTC ATAGTTTCTT	2234
5	CCCTAAGTTT CTTACCGCAT GCTTTCAGAC TGGGCTCTTC TTTGTTCTCT TGCTGAGGAT	2294
	CTTATTTTAA ATGCAAGTCA CACCTATTCT GCAACTGCAG GTCAGAAATG GTTTCACAGT	2354
	GGGGTGCCAG GAAGCAGGGA AGCTGCAGGA GCCAGTTCTA CTGGGGTGCG TGAATGGAGG	2414
10	TGATGGCAGA CACTTTTACT GAATGTGGGT CTTTTTTTGT GATTATTCTA G TTA TCT Leu Ser	2471
	CCA GAA GAA GAA GAG AAA AGG AGA ATC CGA AGG GAA AGG AAT AAG ATG Pro Glu Glu Glu Glu Lys Arg Arg Ile Arg Arg Glu Arg Asn Lys Met 135 140 145	2519
15	GCT GCA GCC AAA TGC CGC AAC CGG AGG AGG GAG CTG ACT GAT ACA CTC Ala Ala Ala Lys Cys Arg Asn Arg Arg Arg Glu Leu Thr Asp Thr Leu 150 155 160 165	2567
	CAA GCG GTAGGTACTC TGTGGGTTGC TCCTTTTAA AACTTAAGGG AAAGTTGGAG Gln Ala	2623
20	ATTGAGCATA AGGGCCCTTG AGTAAGACTG TGTCTTATGC TTTCCTTTAT CCCTCTGTAT	2683
	ACAG GAG ACA GAC CAA CTA GAA GAT GAG AAG TCT GCT TTG CAG ACC GAG Glu Thr Asp Gln Leu Glu Asp Glu Lys Ser Ala Leu Gln Thr Glu 170 175 180	2732
25	ATT GCC AAC CTG CTG AAG GAG AAG GAA AAA CTA GAG TTC ATC CTG GCA Ile Ala Asn Leu Leu Lys Glu Lys Glu Lys Leu Glu Phe Ile Leu Ala 185 190 195	2780
	GCT CAC CGA CCT GCC TGC AAG ATC CCT GAT GAC CTG GGC TTC CCA GAA Ala His Arg Pro Ala Cys Lys Ile Pro Asp Asp Leu Gly Phe Pro Glu 200 205 210	2828
30	GAG ATG TCT GTG GCT TCC CTT GAT CTG ACT GGG GGC CTG CCA GAG GTT Glu Met Ser Val Ala Ser Leu Asp Leu Thr Gly Gly Leu Pro Glu Val 215 220 225 230	2876
	GCC ACC CCG GAG TCT GAG GAG GCC TTC ACC CTG CCT CTC CTC AAT GAC Ala Thr Pro Glu Ser Glu Glu Ala Phe Thr Leu Pro Leu Leu Asn Asp 235 240 245	2924
35	CCT GAG CCC AAG CCC TCA GTG GAA CCT GTC AAG AGC ATC AGC AGC ATG Pro Glu Pro Lys Pro Ser Val Glu Pro Val Lys Ser Ile Ser Ser Met 250 255 260	2972
40	GAG CTG AAG ACC GAG CCC TTT GAT GAC TTC CTG TTC CCA GCA TCA TCC Glu Leu Lys Thr Glu Pro Phe Asp Asp Phe Leu Phe Pro Ala Ser Ser 265 270 275	3020
	AGG CCC AGT GGC TCT GAG ACA GCC CGC TCC GTG CCA GAC ATG GAC CTA Arg Pro Ser Gly Ser Glu Thr Ala Arg Ser Val Pro Asp Met Asp Leu 280 285 290	3068
45	TCT GGG TCC TTC TAT GCA GCA GAC TGG GAG CCT CTG CAC AGT GGC TCC Ser Gly Ser Phe Tyr Ala Ala Asp Trp Glu Pro Leu His Ser Gly Ser 295 300 305 310	3116
50	CTG GGG ATG GGG CCC ATG GCC ACA GAG CTG GAG CCC CTG TGC ACT CCG Leu Gly Met Gly Pro Met Ala Thr Glu Leu Glu Pro Leu Cys Thr Pro 315 320 325	3164

EP 0 599 077 A2

	GTG GTC ACC TGT ACT CCC AGC TGC ACT GCT TAC ACG TCT TCC TTC GTC	3212
	Val Val Thr Cys Thr Pro Ser Cys Thr Ala Tyr Thr Ser Ser Phe Val	
	330 335 340	
5	TTC ACC TAC CCC GAG GCT GAC TCC TTC CCC AGC TGT GCA GCT GCC CAC	3260
	Phe Thr Tyr Pro Glu Ala Asp Ser Phe Pro Ser Cys Ala Ala Ala His	
	345 350 355	
	CGC AAG GGC AGC AGC AGC AAT GAG CCT TCC TCT GAC TCG CTC AGC TCA	3308
	Arg Lys Gly Ser Ser Ser Asn Glu Pro Ser Ser Asp Ser Leu Ser Ser	
	360 365 370	
10	CCC ACG CTG CTG GCC CTG TGAGGGGGCA GGGAGGGGA GGCAGCCGGC	3356
	Pro Thr Leu Leu Ala Leu	
	375 380	
	ACCCACAAGT GCCACTGCCC GAGCTGGTGC ATTACAGAGA GGAGAAACAC ATCTTCCCTA	3416
15	GAGGGTTCCT GTAGACCTAG GGAGGACCTT ATCTGTGCGT GAAACACACC AGGCTGTGGG	3476
	CCTCAAGGAC TTGAAAGCAT CCATGTGTGG ACTCAAGTCC TTACCTCTTC CGGAGATGTA	3536
	GCAAAAACGCA TGGAGTGTGT ATTGTTCCCA GTGACACTTC AGAGAGCTGG TAGTTAGTAG	3596
20	CATGTTGAGC CAGGCCTGGG TCTGTGTCTC TTTTCTCTTT CTCCCTAGTC TTCTCATAGC	3656
	ATTAACATAAT CTATTGGGTT CATTATTGGA ATTAACCTGG TGCTGGATAT TTTCAAATTG	3716
	TATCTAGTGC AGCTGATTTT AACAATAACT ACTGTGTTCC TGGCAATAGT GTGTTCTGAT	3776
25	TAGAAATGAC CAATATTATA CTAAGAAAAG ATACGACTTT ATTTTCTGGT AGATAGAAAT	3836
	AAATAGCTAT ATCCATGTAC TGTAGTTTTT CTCAACATC AATGTTCAAT GTAATGTTAC	3896
	TGATCATGCA TTGTTGAGGT GGTCTGAATG TTCTGACATT AACAGTTTTT CATGAAAACG	3956
	TTTTATTGTC TTTTAAATT ATTATTAAAG ATGGATTCTC AGATATTTAT ATTTTATTAT	4016
30	TATTTTTTTC TACCTTGAGG TCTTTTGACA TGTGGAAAGT GAATTTGAAT CAAAAATTTA	4076
	AGCATTGTTT GCTTATTGTT CCAAGACATT GTCAATAAAA GCATTTAAGT TGAATGCGAC	4136
	CAACCTTGTC CTCTTTTCAT TCTGGAAGTC TTGTAAGTTT CTGAAAGGTA TTATTGGAGA	4196
35	CCAGTTTGTC AAGAAGGGTA GCTGCTGGAG GGGGACACAC CCTCTGTCTG ATCCCTTATC	4256
	AAAGAGGACA AGGAAACTAT AGAGCTGATT TTAGAATATT TTACAAATAC ATGCCTTCCA	4316
	TTGGAATGCT AAGATTTTCT ACTGCTTCTG GGGACGGGAA ACCGCTGTGT AACAGCTTTT	4376
40	GTGGGAATAC ATTTTTTCTG TTTCAGTACT CGCAGGGGGA AATATTTAAA TTTTGTGTG	4436
	CTAATATTAA ATTCAGATGT TTTGATCTTA AAGGAACCCT TTAAGCAAAC AGAACCTAGC	4496
	TTTGATACAGA CTATTTTAAC TTTTATTCT CACAAAATCA CGTGGAGGGT TATTCTACTT	4556
45	CAAAGATGAG CAAATGAAG AATGGTTAGA ATAAACAAC TTCTTGATAT TCCGTTATCG	4616
	GCATTAGAAT CTTCTGCTC GTTATCGTAT CCAGCAGGCT GAACTGCCTC TTGATACTTG	4676
	GTTAAAAAAA ATTTTCAGGC CGGGCCGGCT GGCCCATGCC TGTAATCCTA GCACTTTGGG	4736
	AGGCCGAGGC AGGCCGATCA CCTGAGGTCG GGAGTTCGAG ACCAGCCTGA CCAACATGGA	4796
50	GAAACCCCGT CTTTACTAAA AATACAAAAT TAGCCTGGTG TGCTGGTGCA TGCTGTAAAT	4856

CCTAGCTACT TGAGAGGCTG AGACAGGAAA ATCACTTGAA CTCGGGAGGC GGATGTTGCA 4916
 GCGAACTGAG ATTGCGCCAT TGCACTCCAG CCTGGGCAAC AAGATTGAAA CTCTGTTTAA 4976
 5 AAAAAAAGT TTTCACATAAT GTGTACATTT TTTTGTACTC TTTTATTCTC GAAAGGGAAG 5036
 GAGGGCTATT GCCCTATCCC TTATTAATAA ATGCATTGTG GTTCTGTTT TCTCTAATAC 5096
 CATATGCCCT TCATTCAATT TATAGTGGGC GGAAGTGGGG GAGAAAAAGT TGCTCAGAAA 5156
 TCAAAAGATA TCTCAAACAG CACAAATAAT GGCTGATCGT TCTGCAACA AAAAGTTACA 5216
 10 TAATAGCTCA AGAAGGAGAA GTCAACATGA CTCTGAACAA GCTTTAACTT AGAAACTTTA 5276
 TCATCTTAAG GAAGAACGTG ACCTTTGTCC AGGACGTCTC TGGTAATGGG GCACCTACAC 5336
 ACACATGCAC ACGTACAAAC CACAGGGAAG GGAGACCGCC CTTCTGCCTC TGCTCGCGAG 5396
 15 TATCAGCGAG GCACCATGCA CTATGTTTTT ACACACACTG GGTGGAAGAA GAGCTTCAGC 5456
 GCCAGTCTTC TAATGCTTTG GTGATAATGA AAATCACTGG GTGCTTATGG GGTGTCATAT 5516
 TCAATCGAGT TAAAAGTTTT AATTCAAAT GACAGTTTTA CTGAGGTTGA TGTCTCGTC 5576
 20 TATGATATCT CTGCCCTCC CATAAAATG GACATTTAAA AGCAACTTAC CGCTCTTTAG 5636
 ATCACTCCTA TATCACACAC CACTTGGGGT GCTGTTTCTG CTAGACTTGT GATGACAGTG 5696
 GCCTTAGGAT CCCTGTTTGC TGTTCAAAGG GCAAATATTT TATAGCCTTT AAATATACCT 5756
 25 AAATAAATA CAGAATTAAT ATAATAACA AACACCTGGT CTGAAATAAC AAGGTGATCT 5816
 ACCCTGGAAG GAACCCAGCT GGTGGGCCAG GAGCGGTGGC TCACACCTGT AATCCAGCA 5876
 CTTTGGGAGG CTGAGACAGG AGGATCACTG GAGTCCAGGA GTTTGAGACC AGCCTGGGCA 5936
 ACATGGCAAA ACCCAGTGTG CTTCTGTTGT CCCAGCTACA CTACTCAGGA GGCTGAGGCA 5996
 30 GGAGTATGAC TTGAGCCTGG GAGGGGGAGG TTGCAGAGAA CTGATATTGC ACCACCACTG 6056
 CACTCCAGCC TGGGTGACAC AGCAAAACCC TATCTCAAAA AAAAAAAAAA AAAAAAGGAA 6116
 CCCAGCTGGT TCCTGTAGGT GTGCAATAAT AACAAACCAGA GGAAGAAAAG GAAGACGATT 6176
 35 TCCCAGATGA AGAAGGGCAG CTGGACCTTC GGAC 6210

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 380 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Met Phe Ser Gly Phe Asn Ala Asp Tyr Glu Ala Ser Ser Ser Arg
 1 5 10 15
 Cys Ser Ser Ala Ser Pro Ala Gly Asp Ser Leu Ser Tyr Tyr His Ser
 20 25 30

EP 0 599 077 A2

Pro Ala Asp Ser Phe Ser Ser Met Gly Ser Pro Val Asn Ala Gln Asp
35 40 45

Phe Cys Thr Asp Leu Ala Val Ser Ser Ala Asn Phe Ile Pro Thr Val
50 55 60

Thr Ala Ile Ser Thr Ser Pro Asp Leu Gln Trp Leu Val Gln Pro Ala
65 70 75 80

Leu Val Ser Ser Val Ala Pro Ser Gln Thr Arg Ala Pro His Pro Phe
85 90 95

Gly Val Pro Ala Pro Ser Ala Gly Ala Tyr Ser Arg Ala Gly Val Val
100 105 110

Lys Thr Met Thr Gly Gly Arg Ala Gln Ser Ile Gly Arg Arg Gly Lys
115 120 125

Val Glu Gln Leu Ser Pro Glu Glu Glu Lys Arg Arg Ile Arg Arg
130 135 140

Glu Arg Asn Lys Met Ala Ala Ala Lys Cys Arg Asn Arg Arg Arg Glu
145 150 155 160

Leu Thr Asp Thr Leu Gln Ala Glu Thr Asp Gln Leu Glu Asp Glu Lys
165 170 175

Ser Ala Leu Gln Thr Glu Ile Ala Asn Leu Leu Lys Glu Lys Glu Lys
180 185 190

Leu Glu Phe Ile Leu Ala Ala His Arg Pro Ala Cys Lys Ile Pro Asp
195 200 205

Asp Leu Gly Phe Pro Glu Glu Met Ser Val Ala Ser Leu Asp Leu Thr
210 215 220

Gly Gly Leu Pro Glu Val Ala Thr Pro Glu Ser Glu Glu Ala Phe Thr
225 230 235 240

Leu Pro Leu Leu Asn Asp Pro Glu Pro Lys Pro Ser Val Glu Pro Val
245 250 255

Lys Ser Ile Ser Ser Met Glu Leu Lys Thr Glu Pro Phe Asp Asp Phe
260 265 270

Leu Phe Pro Ala Ser Ser Arg Pro Ser Gly Ser Glu Thr Ala Arg Ser
275 280 285

Val Pro Asp Met Asp Leu Ser Gly Ser Phe Tyr Ala Ala Asp Trp Glu
290 295 300

Pro Leu His Ser Gly Ser Leu Gly Met Gly Pro Met Ala Thr Glu Leu
305 310 315 320

Glu Pro Leu Cys Thr Pro Val Val Thr Cys Thr Pro Ser Cys Thr Ala
325 330 335

Tyr Thr Ser Ser Phe Val Phe Thr Tyr Pro Glu Ala Asp Ser Phe Pro
340 345 350

Ser Cys Ala Ala Ala His Arg Lys Gly Ser Ser Ser Asn Glu Pro Ser
355 360 365

Ser Asp Ser Leu Ser Ser Pro Thr Leu Leu Ala Leu
370 375 380

(2) INFORMATION FOR SEQ ID NO:8:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCAGTCAGG TCAGGGGTGG GGAAGCCCAG GGCTGGGGAT TCCCCATCT

49

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(i) APPLICANT:

(A) NAME: Yeda Research and Development Co., Ltd.
 (B) STREET: P.O. Box 95
 (C) CITY: Rehovot
 (E) COUNTRY: Israel
 10 (F) POSTAL CODE (ZIP): 76100

(ii) TITLE OF INVENTION: Anti-Metastatic Vaccine

(iii) NUMBER OF SEQUENCES: 8

15

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

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(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP 93 11 7519.4

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 968,415
 (B) FILING DATE: 29-OCT-1992

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3135 base pairs
 (B) TYPE: nucleic acid
 30 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(ix) FEATURE:

(A) NAME/KEY: mRNA
 (B) LOCATION: 1..3135

(ix) FEATURE:

(A) NAME/KEY: CDS
 40 (B) LOCATION: 917..1918

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTGAGTGTGC GAGAGACAGC CTGGCAGGAG AGCGCTCAGG CAGACAGACA GACAGACGGA 60
 45 CGGACTTGGC CAACCCGGTC GGCCGCGGAC TCCGGACTGT TCATCCGTTT GTCTTCATTT 120
 TCTCACCAAC TGCTTGGATC CAGCGCCCGC GGCTCCTGCA CCGGTATTTT GGGGAGCATT 180
 TGGAGAGTCC CTTCTCCCGC CTTCCACGGA GAAGAAGCTC ACAAGTCCGG GCGCTGCTGA 240
 50

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EP 0 599 077 A2

	CAGCATCGAG AGCGGCTCCC GACCGCGCGA GGAAATAGGC GAGCGGCTAC CGGCCAGCAA	300
	CTTTCCTGAC CCAGAGGACC GGTAACAAGT GGCCGGGAGC GAACTTTTGC AAATCTCTTC	360
5	TGCGCCTTAA GGCTGCCACC GAGACTGTAA AGAAAAGGGA GAAGAGGAAC CTATACTCAT	420
	ACCAGTTTCGC ACAGGCGGCT GAAGTTGGGC GAGCGCTAGC CGCGGCTGCC TAGCGTCCCC	480
	CTCCCCCTCA CAGCGGAGGA GGGGACAGTT GTTGGAGGCC GGGCGGCAGA GCCCGATCGC	540
10	GGGCTTCCAC CGAGAATTCC GTGACGACTG GTCAGCACCG CCGGAGAGCC GCTGTTGCTG	600
	GGACTGGTCT GCGGGCTCCA AGGAACCGCT GCTCCCCGAG AGCGCTCCGT GAGTGACCGC	660
	GACTTTTCAA AGCTCGGCAT CGCGCGGGAG CCTACCAACG TGAGTGCTAG CGGAGTCTTA	720
15	ACCTGCGCT CCTGGAGCG AACTGGGGAG GAGGGCTCAG GGGGAAGCAC TGCCGTCTGG	780
	AGCGCACGCT CCTAAACAAA CTTTGTTACA GAAGCAGGGA CGCGCGGGTA TCCCCCGCT	840
	TCCCGGCGCG CTGTTGCGGC CCCGAACTT CTGCGCACAG CCCAGGCTAA CCCC GCGTGA	900
20	AGTGACGGAC CGTTCT ATG ACT GCA AAG ATG GAA ACG ACC TTC TAC GAC Met Thr Ala Lys Met Glu Thr Thr Phe Tyr Asp 1 5 10	949
	GAT GCC CTC AAC GCC TCG TTC CTC CAG TCC GAG AGC GGT GCC TAC GGC Asp Ala Leu Asn Ala Ser Phe Leu Gln Ser Glu Ser Gly Ala Tyr Gly 15 20 25	997
25	TAC AGT AAC CCT AAG ATC CTA AAA CAG AGC ATG ACC TTG AAC CTG GCC Tyr Ser Asn Pro Lys Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala 30 35 40	1045
30	GAC CCG GTG GGC AGT CTG AAG CCG CAC CTC CGC GCC AAG AAC TCG GAC Asp Pro Val Gly Ser Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp 45 50 55	1093
35	CTT CTC ACG TCG CCC GAC GTC GGG CTG CTC AAG CTG GCG TCG CCG GAG Leu Leu Thr Ser Pro Asp Val Gly Leu Leu Lys Leu Ala Ser Pro Glu 60 65 70 75	1141
	CTG GAG CGC CTG ATC ATC CAG TCC AGC AAT GGG CAC ATC ACC ACT ACA Leu Glu Arg Leu Ile Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr 80 85 90	1189
40	CCG ACC CCC ACC CAG TTC TTG TGC CCC AAG AAC GTG ACC GAC GAG CAG Pro Thr Pro Thr Gln Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln 95 100 105	1237
	GAG GGC TTC GCC GAG GGC TTC GTG CGC GCC CTG GCT GAA CTG CAT AGC Glu Gly Phe Ala Glu Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser 110 115 120	1285
45	CAG AAC ACG CTT CCC AGT GTC ACC TCC GCG GCA CAG CCG GTC AGC GGG Gln Asn Thr Leu Pro Ser Val Thr Ser Ala Ala Gln Pro Val Ser Gly 125 130 135	1333

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EP 0 599 077 A2

	GCG	GGC	ATG	GTG	GCT	CCC	GCG	GTG	GCC	TCA	GTA	GCA	GGC	GCT	GGC	GGC	1381
	Ala	Gly	Met	Val	Ala	Pro	Ala	Val	Ala	Ser	Val	Ala	Gly	Ala	Gly	Gly	
	140					145					150					155	
5	GGT	GGT	GGC	TAC	AGC	GCC	AGC	CTG	CAC	AGT	GAG	CCT	CCG	GTC	TAC	GCC	1429
	Gly	Gly	Gly	Tyr	Ser	Ala	Ser	Leu	His	Ser	Glu	Pro	Pro	Val	Tyr	Ala	
					160					165					170		
	AAC	CTC	AGC	AAC	TTC	AAC	CCG	GGT	GCG	CTG	AGC	AGC	GGC	GGT	GGG	GCG	1477
	Asn	Leu	Ser	Asn	Phe	Asn	Pro	Gly	Ala	Leu	Ser	Ser	Gly	Gly	Gly	Ala	
10					175					180					185		
	CCC	TCC	TAT	GGC	GCG	GCC	GGG	CTG	GCC	TTT	CCC	TCG	CAG	CCG	CAG	CAG	1525
	Pro	Ser	Tyr	Gly	Ala	Ala	Gly	Leu	Ala	Phe	Pro	Ser	Gln	Pro	Gln	Gln	
			190					195					200				
15	CAG	CAG	CAG	CCG	CCT	CAG	CCG	CCG	CAC	CAC	TTG	CCC	CAA	CAG	ATC	CCG	1573
	Gln	Gln	Gln	Pro	Pro	Gln	Pro	Pro	His	His	Leu	Pro	Gln	Gln	Ile	Pro	
			205				210					215					
	GTG	CAG	CAC	CCG	CGG	CTG	CAA	GCC	CTG	AAG	GAA	GAG	CCG	CAG	ACC	GTG	1621
	Val	Gln	His	Pro	Arg	Leu	Gln	Ala	Leu	Lys	Glu	Glu	Pro	Gln	Thr	Val	
20						225					230					235	
	CCG	GAG	ATG	CCG	GGA	GAG	ACG	CCG	CCC	CTG	TCC	CCT	ATC	GAC	ATG	GAG	1669
	Pro	Glu	Met	Pro	Gly	Glu	Thr	Pro	Pro	Leu	Ser	Pro	Ile	Asp	Met	Glu	
					240					245					250		
25	TCT	CAG	GAG	CGG	ATC	AAG	GCA	GAG	AGG	AAG	CGC	ATG	AGG	AAC	CGC	ATT	1717
	Ser	Gln	Glu	Arg	Ile	Lys	Ala	Glu	Arg	Lys	Arg	Met	Arg	Asn	Arg	Ile	
				255					260					265			
	GCC	GCC	TCC	AAG	TGC	CGG	AAA	AGG	AAG	CTG	GAG	CGG	ATC	GCT	CGG	CTA	1765
	Ala	Ala	Ser	Lys	Cys	Arg	Lys	Arg	Lys	Leu	Glu	Arg	Ile	Ala	Arg	Leu	
30				270				275					280				
	GAG	GAA	AAA	GTG	AAA	ACC	TTG	AAA	GCG	CAA	AAC	TCC	GAG	CTG	GCA	TCC	1813
	Glu	Glu	Lys	Val	Lys	Thr	Leu	Lys	Ala	Gln	Asn	Ser	Glu	Leu	Ala	Ser	
			285				290					295					
35	ACG	GCC	AAC	ATG	CTC	AGG	GAA	CAG	GTG	GCA	CAG	CTT	AAG	CAG	AAA	GTC	1861
	Thr	Ala	Asn	Met	Leu	Arg	Glu	Gln	Val	Ala	Gln	Leu	Lys	Gln	Lys	Val	
						305					310					315	
	ATG	AAC	CAC	GTT	AAC	AGT	GGG	TGC	CAA	CTC	ATG	CTA	ACG	CAG	CAG	TTG	1909
	Met	Asn	His	Val	Asn	Ser	Gly	Cys	Gln	Leu	Met	Leu	Thr	Gln	Gln	Leu	
40					320					325					330		
	CAA	ACG	TTT	TGAGAACAGA	CTGTCAGGGC	TGAGGGGCAA	TGGAAGAAAA										1958
	Gln	Thr	Phe														
45	AAAATAACAG	AGACAAACTT	GAGAACTTGA	CTGGTTGCGA	CAGAGAAAAA	AAAAGTGTCC											2018
	GAGTACTGAA	GCCAAGGGTA	CACAAGATGG	ACTGGGTTGC	GACCTGACGG	CGCCCCCAGT											2078
	GTGCTGGAGT	GGGAAGGACG	TGGCGCGCCT	GGCTTTGGCG	TGGAGCCAGA	GAGCAGCGGC											2138
50	CTATTGGCCG	GCAGACTTTG	CGGACGGGCT	GTGCCCGCGC	GCGACCAGAA	CGATGGACTT											2198

EP 0 599 077 A2

TTCGTTAACA TTGACCAAGA ACTGCATGGA CCTAACATTC GATCTCATTC AGTATTAAAG 2258
 GGGGGTGGGA GGGGTTACAA ACTGCAATAG AGACTGTAGA TTGCTTCTGT AGTGCTCCTT 2318
 5 AACACAAAGC AGGGAGGGCT GGAAGGGGG GGAGGCTTGT AAGTGCCAGG CTAGACTGCA 2378
 GATGAACTCC CCTGGCCTGC CTCTCTCAAC TGTGTATGTA CATATATATT TTTTTTTAAT 2438
 TTGATGAAAG CTGATTACTG TCAATAAACA GCTTCCTGCC TTTGTAAGTT ATTCCATGTT 2498
 10 TGTGTTGTTG GGTGTCCTGC CCAGTGTTTG TAAATAAGAG ATTTGAAGCA TTCTGAGTTT 2558
 ACCATTTGTA ATAAAGTATA TAATTTTTTT ATGTTTTGTT TCTGAAAATT TCCAGAAAGG 2618
 ATATTTAAGA AAATACAATA AACTATTGAA AAGTAGCCCC CAACCTCTTT GCTGCATTAT 2678
 15 CCATAGATAA TGATAGCTAG ATGAAGTGAC AGCTGAGTGC CCCCATATA CTAGGGTGAA 2738
 AGCTGTGTCC CCTGTCTGAT TTGTAGGAAT AGATACCCTG CATGCTATCA TTGGCTCATA 2798
 CTCTCTCCCC CGGCAACACA CAAGTCCAGA CTGTACACCA GAAGATGGTG TGGTGTCTCT 2858
 20 TAAGGCTGGA AGAAGGGCTG TTGCAAGGGG AGAGGGTCAG CCCGCTGGAA AGCAGACACT 2918
 TTGGTTGAAA GCTGTATGAA GTGGCATGTG CTGTGATCAT TTATAATCAT AGGAAAGATT 2978
 TAGTAATTAG CTGTTGATTC TCAAAGCAGG GACCCATGGA AGTTTTTAAC AAAAGGTGTC 3038
 25 TCCTTCCAAC TTTGAATCTG ACAACTCCTA GAAAAAGATG ACCTTTGCTT GTGCATATTT 3098
 ATAATAGCGT TCGTTATCAC AATAAATGTA TTCAAAT 3135

(2) INFORMATION FOR SEQ ID NO: 2:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 334 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 35 (ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
 Met Thr Ala Lys Met Glu Thr Thr Phe Tyr Asp Asp Ala Leu Asn Ala
 1 5 10 15
 40 Ser Phe Leu Gln Ser Glu Ser Gly Ala Tyr Gly Tyr Ser Asn Pro Lys
 20 25 30
 Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala Asp Pro Val Gly Ser
 35 40 45
 45 Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp Leu Leu Thr Ser Pro
 50 55 60
 Asp Val Gly Leu Leu Lys Leu Ala Ser Pro Glu Leu Glu Arg Leu Ile
 65 70 75 80

EP 0 599 077 A2

Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr Pro Thr Pro Thr Gln
85 90 95

5 Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln Glu Gly Phe Ala Glu
100 105 110

Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser Gln Asn Thr Leu Pro
115 120 125

10 Ser Val Thr Ser Ala Ala Gln Pro Val Ser Gly Ala Gly Met Val Ala
130 135 140

Pro Ala Val Ala Ser Val Ala Gly Ala Gly Gly Gly Gly Tyr Ser
145 150 155 160

15 Ala Ser Leu His Ser Glu Pro Pro Val Tyr Ala Asn Leu Ser Asn Phe
165 170 175

Asn Pro Gly Ala Leu Ser Ser Gly Gly Gly Ala Pro Ser Tyr Gly Ala
180 185 190

20 Ala Gly Leu Ala Phe Pro Ser Gln Pro Gln Gln Gln Gln Gln Pro Pro
195 200 205

Gln Pro Pro His His Leu Pro Gln Gln Ile Pro Val Gln His Pro Arg
210 215 220

25 Leu Gln Ala Leu Lys Glu Glu Pro Gln Thr Val Pro Glu Met Pro Gly
225 230 235 240

Glu Thr Pro Pro Leu Ser Pro Ile Asp Met Glu Ser Gln Glu Arg Ile
245 250 255

30 Lys Ala Glu Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys
260 265 270

Arg Lys Arg Lys Leu Glu Arg Ile Ala Arg Leu Glu Glu Lys Val Lys
275 280 285

35 Thr Leu Lys Ala Gln Asn Ser Glu Leu Ala Ser Thr Ala Asn Met Leu
290 295 300

Arg Glu Gln Val Ala Gln Leu Lys Gln Lys Val Met Asn His Val Asn
305 310 315 320

Ser Gly Cys Gln Leu Met Leu Thr Gln Gln Leu Gln Thr Phe
325 330

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3622 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

EP 0 599 077 A2

(ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION: 287..3622

5 (ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION: 289..3622

(ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION: 293..3622

10 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1261..2253

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCCCGGGAGG	GGACCGGGGA	ACAGAGGGCC	GAGAGGCGTG	CGGCAGGGGG	GAGGGTAGGA	60
GAAAGAAGGG	CCCGACTGTA	GGAGGGCAGC	GGAGCATTAC	CTCATCCCGT	GAGCCTCCGC	120
20 GGGCCCAGAG	AAGAATCTTC	TAGGGTGGAG	TCTCCATGGT	GACGGGCGGG	CCCGCCCCCC	180
TGAGAGCGAC	GCGAGCCAAT	GGGAAGGCCT	TGGGGTGACA	TCATGGGCTA	TTTTTAGGGG	240
TTGACTGGTA	GCAGATAAGT	GTTGAGCTCG	GGCTGGATAA	GGGCTCAGAG	TTGCACTGAG	300
25 TGTGGCTGAA	GCAGCGAGGC	GGGAGTGGAG	GTGCGCGGAG	TCAGGCAGAC	AGACAGACAC	360
AGCCAGCCAG	CCAGGTCGGC	AGTATAGTCC	GAATGCAAA	TCTTATTTTC	TTTTACCTT	420
CTCTCTAACT	CCCCAGAGCT	AGCGCCTGTG	GCTCCCGGGC	TGGTGGTTCG	GGAGTGTTCA	480
30 GAGAGCCTTG	TCTCCAGCCG	CCCCCGGGAG	GAGAGCCCTG	CTGCCCAGGC	GCTGTTGACA	540
GCGGCGGAAA	GCAGCGGTAC	CCCACGCGCC	CGCCGGGGGA	CGTCGGCGAG	CGGCTGCAGC	600
AGCAAAGAAC	TTTCCCGGCG	GGGAGGACCG	GAGACAAGTG	GCAGAGTCCC	GGAGCGAACT	660
35 TTTGCAAGCC	TTTCTGCGT	CTTAGGCTTC	TCCACGGCGG	TAAAGACCAG	AAGGCGGCGG	720
AGAGCCACGC	AAGAGAAGAA	GGACGTGCGC	TCAGCTTCGC	TCGCACCGGT	TGTTGAACTT	780
GGGCGAGCGC	GAGCCGCGGC	TGCCGGGCGC	CCCCTCCCCC	TAGCAGCGGA	GGAGGGGACA	840
40 AGTCGTCGGA	GTCCGGGCGG	CCAAGACCCG	CCGCCGGCCG	GCCACTGCAG	GGTCCGCACT	900
GATCCGCTCC	GCGGGGAGAG	CCGCTGCTCT	GGGAAGTGAG	TTCGCCTGCG	GACTCCGAGG	960
AACCGCTGCG	CCCGAAGAGC	GCTCAGTGAG	TGACCGCGAC	TTTTCAAAGC	CGGGTAGCGC	1020
45 GCGCGAGTCG	ACAAGTAAGA	GTGCGGGAGG	CATCTTAATT	AACCCTGCGC	TCCCTGGAGC	1080
GAGCTGGTGA	GGAGGGCGCA	GCGGGGACGA	CAGCCAGCGG	GTGCGTGCGC	TCTTAGAGAA	1140
ACTTTCCCTG	TCAAAGGCTC	CGGGGGGCGC	GGGTGTCCCC	CGCTTGCCAG	AGCCCTGTTG	1200

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EP 0 599 077 A2

	CGGCCCCGAA ACTTGTGCGC GCACGCCAAA CTAACCTCAC GTGAAGTGAC GGACTGTTCT	1260
	ATG ACT GCA AAG ATG GAA ACG ACC TTC TAT GAC GAT GCC CTC AAC GCC Met Thr Ala Lys Met Glu Thr Thr Phe Tyr Asp Asp Ala Leu Asn Ala	1308
5	1 5 10 15	
	TCG TTC CTC CCG TCC GAG AGC GGA CCT TAT GGC TAC AGT AAC CCC AAG Ser Phe Leu Pro Ser Glu Ser Gly Pro Tyr Gly Tyr Ser Asn Pro Lys	1356
	20 25 30	
10	ATC CTG AAA CAG AGC ATG ACC CTG AAC CTG GCC GAC CCA GTG GGG AGC Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala Asp Pro Val Gly Ser	1404
	35 40 45	
	CTG AAG CCG CAC CTC CGC GCC AAG AAC TCG GAC CTC CTC ACC TCG CCC Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp Leu Leu Thr Ser Pro	1452
15	50 55 60	
	GAC GTG GGG CTG CTC AAG CTG GCG TCG CCC GAG CTG GAG CGC CTG ATA Asp Val Gly Leu Leu Lys Leu Ala Ser Pro Glu Leu Glu Arg Leu Ile	1500
	65 70 75 80	
20	ATC CAG TCC AGC AAC GGG CAC ATC ACC ACC ACG CCG ACC CCC ACC CAG Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr Pro Thr Pro Thr Gln	1548
	85 90 95	
	TTC CTG TGC CCC AAG AAC GTG ACA GAT GAG CAG GAG GGG TTC GCC GAG Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln Glu Gly Phe Ala Glu	1596
25	100 105 110	
	GGC TTC GTG CGC GCC CTG GCC GAA CTG CAC AGC CAG AAC ACG CTG CCC Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser Gln Asn Thr Leu Pro	1644
	115 120 125	
30	AGC GTC ACG TCG GCG GCG CAG CCG GTC AAC GGG GCA GGC ATG GTG GCT Ser Val Thr Ser Ala Ala Gln Pro Val Asn Gly Ala Gly Met Val Ala	1692
	130 135 140	
	CCC GCG GTA GCC TCG GTG GCA GGG GGC AGC GGC AGC GGC GGC TTC AGC Pro Ala Val Ala Ser Val Ala Gly Gly Ser Gly Ser Gly Gly Phe Ser	1740
35	145 150 155 160	
	GCC AGC CTG CAC AGC GAG CCG CCG GTC TAC GCA AAC CTC AGC AAC TTC Ala Ser Leu His Ser Glu Pro Pro Val Tyr Ala Asn Leu Ser Asn Phe	1788
	165 170 175	
40	AAC CCA GGC GCG CTG AGC AGC GGC GGC GGG GCG CCC TCC TAC GGC GCG Asn Pro Gly Ala Leu Ser Ser Gly Gly Gly Ala Pro Ser Tyr Gly Ala	1836
	180 185 190	
	GCC GGC CTG GCC TTT CCC GCG CAA CCC CAG CAG CAG CAG CAG CCG CCG Ala Gly Leu Ala Phe Pro Ala Gln Pro Gln Gln Gln Gln Gln Pro Pro	1884
45	195 200 205	
	CAC CAC CTG CCC CAG CAG ATG CCC GTG CAG CAC CCG CGG CTG CAG GCC His His Leu Pro Gln Gln Met Pro Val Gln His Pro Arg Leu Gln Ala	1932
	210 215 220	
50		
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EP 0 599 077 A2

	CTG AAG GAG GAG CCT CAG ACA GTG CCC GAG ATG CCC GGC GAG ACA CCG	1980
	Leu Lys Glu Glu Pro Gln Thr Val Pro Glu Met Pro Gly Glu Thr Pro	
	225 230 235 240	
5	CCC CTG TCC CCC ATC GAC ATG GAG TCC CAG GAG CGG ATC AAG GCG GAG	2028
	Pro Leu Ser Pro Ile Asp Met Glu Ser Gln Glu Arg Ile Lys Ala Glu	
	245 250 255	
	AGG AAG CGC ATG AGG AAC CGC ATC GCT GCC TCC AAG TGC CGA AAA AGG	2076
	Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys Arg Lys Arg	
10	260 265 270	
	AAG CTG GAG AGA ATC GCC CGG CTG GAG GAA AAA GTG AAA ACC TTG AAA	2124
	Lys Leu Glu Arg Ile Ala Arg Leu Glu Glu Lys Val Lys Thr Leu Lys	
	275 280 285	
15	GCT CAG AAC TCG GAG CTG GCG TCC ACG GCC AAC ATG CTC AGG GAA CAG	2172
	Ala Gln Asn Ser Glu Leu Ala Ser Thr Ala Asn Met Leu Arg Glu Gln	
	290 295 300	
	GTG GCA CAG CTT AAA CAG AAA GTC ATG AAC CAC GTT AAC AGT GGG TGC	2220
	Val Ala Gln Leu Lys Gln Lys Val Met Asn His Val Asn Ser Gly Cys	
20	305 310 315 320	
	CAA CTC ATG CTA ACG CAG CAG TTG CAA ACA TTT TGAAGAGAGA CCGTCGGGGG	2273
	Gln Leu Met Leu Thr Gln Gln Leu Gln Thr Phe	
	325 330	
25	CTGAGGGGCA ACGAAGAAAA AAAATAACAC AGAGAGACAG ACTTGAGAAC TTGACAAGTT	2333
	GCGACGGAGA GAAAAAAGAA GTGTCCGAGA ACTAAAGCCA AGGGTATCCA AGTTGGACTG	2393
	GGTTCCGTCT GACGGCGCCC CCAGTGTGCA CGAGTGGGAA GGAAGTGGTC GCGCCCTCCC	2453
30	TTGGCGTGGA GCCAGGGAGC GGCCGCCTGC GGGCTGCCCC GCTTTGCGGA CGGGCTGTCC	2513
	CCGCGCGAAC GGAACGTTGG ACTTTCGTTA ACATTGACCA AGAACTGCAT GGACCTAACA	2573
	TTCGATCTCA TTCAGTATTA AAGGGGGGAG GGGGAGGGGG TTACAACTG CAATAGAGAC	2633
35	TGTAGATTGC TTCTGTAGTA CTCCTTAAGA ACACAAAGCG GGGGAGGGGT TGGGGAGGGG	2693
	CGGCAGGAGG GAGGTTTGTG AGAGCGAGGC TGAGCCTACA GATGAACTCT TTCTGGCCTG	2753
	CTTTCGTTAA CTGTGTATGT ACATATATAT ATTTTCTAAT TTGATTAAAG CTGATTACTG	2813
40	TCAATAAACA GCTTCATGCC TTTGTAAGTT ATTTCTTGTT TGTGTTTGG GGTATCCTGC	2873
	CCAGTGTGTG TTGTAAATAA GAGATTTGGA GCACTCTGAG TTTACCATTG GTAATAAAGT	2933
	ATATAATTTT TTTATGTTTT GTTCTGAAA ATTCCAGAAA GGATATTTAA GAAAAATACAA	2993
45	TAAACTATTG GAAAGTACTC CCCTAACCTC TTTTCTGCAT CATCTGTAGA TCCTAGTCTA	3053
	TCTAGGTGGA GTTGAAAGAG TTAAGAATGC TCGATAAAAT CACTCTCAGT GCTTCTTACT	3113
	ATTAAGCAGT AAAAAGTGTG CTCTATTAGA CTTAGAAATA AATGTACCTG ATGTACCTGA	3173
50	TGCTATGTCA GGCTTCATAC TCCACGCTCC CCCAGCGTAT CTATATGGAA TTGCTTACCA	3233

EP 0 599 077 A2

AAGGCTAGTG CGATGTTTCA GGAGGCTGGA GGAAGGGGGG TTGCAGTGGA GAGGGACAGC 3293
 CCACTGAGAA GTCAAACATT TCAAAGTTTG GATTGCATCA AGTGGCATGT GCTGTGACCA 3353
 5 TTTATAATGT TAGAAATTTT ACAATAGGTG CTTATTCTCA AAGCAGGAAT TGGTGGCAGA 3413
 TTTTACAAAA GATGTATCCT TCCAATTTGG AATCTTCTCT TTGACAATTC CTAGATAAAA 3473
 AGATGGCCTT TGTCTTATGA ATATTTATAA CAGCATTCTG TCACAATAAA TGTATTCAAA 3533
 10 TACCAATAAC AGATCTTGAA TTGCTTCCCT TTACTACTTT TTTGTTCCCA AGTTATATAC 3593
 TGAAGTTTTT ATTTTATAGTT GCTGAGGTT 3622

(2) INFORMATION FOR SEQ ID NO: 4:

15

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 331 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

20

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Thr Ala Lys Met Glu Thr Thr Phe Tyr Asp Asp Ala Leu Asn Ala
 1 5 10 15
 25 Ser Phe Leu Pro Ser Glu Ser Gly Pro Tyr Gly Tyr Ser Asn Pro Lys
 20 25 30
 Ile Leu Lys Gln Ser Met Thr Leu Asn Leu Ala Asp Pro Val Gly Ser
 35 40 45
 30 Leu Lys Pro His Leu Arg Ala Lys Asn Ser Asp Leu Leu Thr Ser Pro
 50 55 60
 Asp Val Gly Leu Leu Lys Leu Ala Ser Pro Glu Leu Glu Arg Leu Ile
 65 70 75 80
 35 Ile Gln Ser Ser Asn Gly His Ile Thr Thr Thr Pro Thr Pro Thr Gln
 85 90 95
 Phe Leu Cys Pro Lys Asn Val Thr Asp Glu Gln Glu Gly Phe Ala Glu
 100 105 110
 40 Gly Phe Val Arg Ala Leu Ala Glu Leu His Ser Gln Asn Thr Leu Pro
 115 120 125
 Ser Val Thr Ser Ala Ala Gln Pro Val Asn Gly Ala Gly Met Val Ala
 130 135 140
 45 Pro Ala Val Ala Ser Val Ala Gly Gly Ser Gly Ser Gly Gly Phe Ser
 145 150 155 160
 Ala Ser Leu His Ser Glu Pro Pro Val Tyr Ala Asn Leu Ser Asn Phe
 165 170 175
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EP 0 599 077 A2

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Asn Pro Gly Ala Leu Ser Ser Gly Gly Gly Ala Pro Ser Tyr Gly Ala
      180                      185                      190
5  Ala Gly Leu Ala Phe Pro Ala Gln Pro Gln Gln Gln Gln Gln Pro Pro
      195                      200                      205
His His Leu Pro Gln Gln Met Pro Val Gln His Pro Arg Leu Gln Ala
      210                      215                      220
10 Leu Lys Glu Glu Pro Gln Thr Val Pro Glu Met Pro Gly Glu Thr Pro
      225                      230                      235
Pro Leu Ser Pro Ile Asp Met Glu Ser Gln Glu Arg Ile Lys Ala Glu
      245                      250                      255
15 Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys Arg Lys Arg
      260                      265                      270
Lys Leu Glu Arg Ile Ala Arg Leu Glu Glu Lys Val Lys Thr Leu Lys
      275                      280                      285
20 Ala Gln Asn Ser Glu Leu Ala Ser Thr Ala Asn Met Leu Arg Glu Gln
      290                      295                      300
Val Ala Gln Leu Lys Gln Lys Val Met Asn His Val Asn Ser Gly Cys
      305                      310                      315                      320
25 Gln Leu Met Leu Thr Gln Gln Leu Gln Thr Phe
      325                      330

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(2) INFORMATION FOR SEQ ID NO: 5:

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30 (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 3548 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

35 (ix) FEATURE:
    (A) NAME/KEY: TATA_signal
    (B) LOCATION: 101..106

40 (ix) FEATURE:
    (A) NAME/KEY: polyA_signal
    (B) LOCATION: 3493..3498

45 (ix) FEATURE:
    (A) NAME/KEY: exon
    (B) LOCATION: 284..424

    (ix) FEATURE:
    (A) NAME/KEY: exon
    (B) LOCATION: 1179..1430

50 (ix) FEATURE:
    (A) NAME/KEY: exon
    (B) LOCATION: 1836..1943

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EP 0 599 077 A2

(ix) FEATURE:

(A) NAME/KEY: exon
(B) LOCATION: 2061..2702

(ix) FEATURE:

(A) NAME/KEY: precursor_RNA
(B) LOCATION: 133..2702

(ix) FEATURE:

(A) NAME/KEY: intron
(B) LOCATION: 425..1178

(ix) FEATURE:

(A) NAME/KEY: intron
(B) LOCATION: 1431..1835

(ix) FEATURE:

(A) NAME/KEY: intron
(B) LOCATION: 1944..2060

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

20	GAGTTGACGA CAGAGCGCCC GCAGAGGGCC TTGGGGCGCG CTTCCCCCCC CTTCCAGTTC	60
	CGCCCAGTGA CGTAGGAAGT CCATCCATTC ACAGCGCTTC TATAAAGGCG CCAGCTGAGG	120
	CGCCTACTAC TCCAACCGCG ACTGCAGCGA GCAACTGAGA AGACTGGATA GAGCCGGCGG	180
25	TTCCGCGAAC GAGCAGTGAC CGCGCTCCCA CCCAGCTCTG CTCTGCAGCT CCCACCAGTG	240
	TCTACCCCTG GACCCCTTGC CGGGCTTTCC CCAAACCTCG ACCATGATGT TCTCGGGTTT	300
	CAACGCCGAC TACGAGGCGT CATCCTCCCG CTGCAGTAGC GCCTCCCCGG CCGGGGACAG	360
30	CCTTTCCTAC TACCATTCCC CAGCCGACTC CTTCTCCAGC ATGGGCTCTC CTGTCAACAC	420
	ACAGGTGAGT TTGGCTTTGT GTAGCCGCCA GGTCCGCGCT GAGGGTCGCC GTGGAGGAGA	480
	CACTGGGGTG TGA CTG CAG GGGCGGGGG GTCTTCCTTT TCGCTCTGG AGGGAGACTG	540
35	GCGCGGTCAG AGCAGCCTTA GCCTGGGAAC CCAGGACTTG TCTGAGCGCG TGCACACTTG	600
	TCATAGTAAG ACTTAGTGAC CCCTTCCCGC GCGGCAGGTT TATTCTGAGT GGCCTGCCTG	660
	CATTCTTCTC TCGGCCGACT TGTTTCTGAG ATCAGCCGGG GCCAACAAGT CTCGAGCAAA	720
40	GAGTCGCTAA CTAGAGTTTG GGAGGCGGCA AACC GCGGCA ATCCCCCTC CCGGGG CAGC	780
	CTGGAGCAGG GAGGAGGGAG GAGGGAGGAG GGTGCTGCGG GCGGGTGTGT AAGGCAGTTT	840
	CATTGATAAA AAGCGAGTTC ATTCTGGAGA CTCCGAGCA GCGCCTGCGT CAGCGCAGAC	900
45	GTCAGGGATA TTTATAACAA ACCCCCTTTC GAGCGAGTGA TGCCGAAGGG ATAACGGGAA	960
	CGCAGCAGTA GGATGGAGGA GAAAGGCTGC GCTGCGGAAT TCAAGGGAGG ATATTGGGAG	1020
	AGCTTTTATC TCCGATGAGG TGCATACAGG AAGACATAAG CAGTCTCTGA CCGGAATGCT	1080

EP 0 599 077 A2

	TCTCTCTCCC	TGCTTCATGC	GACACTAGGG	CCACTTGCTC	CACCTGTGTC	TGGAACCTCC	1140
	TCGCTCACCT	CCGCTTTCCT	CTTTTTGTTT	TGTTTCAGGA	CTTTTGCGCA	GATCTGTCCG	1200
5	TCTCTAGTGC	CAACTTTATC	CCCACGGTGA	CAGCCATCTC	CACCAGCCCA	GACCTGCAGT	1260
	GGCTGGTGCA	GCCCACTCTG	GTCTCCTCCG	TGGCCCCATC	GCAGACCAGA	GCGCCCCATC	1320
	CTTACGGACT	CCCCACCCAG	TCTGCTGGGG	CTTACGCCAG	AGCGGGAATG	GTGAAGACCG	1380
10	TGTCAGGAGG	CAGAGCGCAG	AGCATCGGCA	GAAGGGGCAA	AGTAGAGCAG	GTGAGCAGCG	1440
	ATTCTGGACC	TTTGTGGGCT	GGGGGGGGGG	GGGGGGGCGG	AGACTGACGC	ACAGACCACA	1500
	CAACAGAGAA	GGGACGCTAC	TGACTGCACT	TCCTGACCAG	GAGCTGTGGC	TGCTAGCCCT	1560
15	TTCCCTCCCT	TGTCAGATTT	TGACAGTTGG	ACCCAAGACA	AACTCTAGAC	AGTTTCCCTG	1620
	ACAGCTTCCT	ACTTCATTCT	CTAGCCGGGG	AGCTTCTTTG	TTCCCTGCT	AAAGATCTCA	1680
	CTTTAAATGC	AAATCACACT	CTGCCTGCCA	ACTGCAGGTT	AGAAAACTG	CTTCACCGAG	1740
20	AGGTGCGGGT	GCTGTAGGAG	CCAGTTTCAC	TGGGGTGACT	GAATGGAGGT	GACACTAGAC	1800
	AACCTTAACT	GAATGTTGGT	CCTTTTCTTC	TATAGCTATC	TCCTGAAGAG	GAAGAGAAAC	1860
	GGAGAATCCG	AAGGGAACGG	AATAAGATGG	CTGCAGCCAA	GTGCCGGAAT	CGGAGGAGGG	1920
25	AGCTGACAGA	TACACTCCAA	GCGGTAGGTT	GAACCAGCTG	CTGCTCCTGA	AACTTTATTA	1980
	AAGTTGGAGC	TTGGGACTAT	GGGCGCAGGG	TCCTTGAGCA	TGCCCGTGTC	TTATGCTTTC	2040
	TTATATCTCT	CCCTATGCAG	GAGACAGATC	AAC'TTGAAGA	TGAGAAGTCT	GCGTTGCAGA	2100
30	CTGAGATTGC	CAATCTGCTG	AAAGAGAAGG	AAAAACTGGA	GTTTATTTTG	GCAGCCCACC	2160
	GACCTGCCTG	CAAGATCCCC	GATGACCTTG	GCTTCCCAGA	GGAGATGTCT	GTGGCCTCCC	2220
	TGGATTTGAC	TGGAGGTCTG	CCTGAGGCTT	CCACCCAGCA	GTCTGAGGAG	GCCTTCACCC	2280
35	TGCCCCTTCT	CAACGACCCT	GAGCCCAAGC	CATCCTTGGA	GCCAGTCAAG	AGCATCAGCA	2340
	ACGTGGAGCT	GAAGGCAGAA	CCCTTTGATG	ACTTCTTGTT	TCCGGCATCA	TCTAGGCCCA	2400
	GTGGCTCAGA	GACCTCCCGC	TCTGTGCCAG	ATGTGGACCT	GTCCGGTTCC	TTCTATGCAG	2460
40	CAGACTGGGA	GCCTCTGCAC	AGCAATTCCCT	TGGGGATGGG	GCCCATGGTC	ACAGAGCTGG	2520
	AGCCCCGTGT	TACTCCCGTG	GTCACCTGTA	CTCCGGGCTG	CACTACTTAC	ACGTCTTCCT	2580
	TTGTCTTCAC	CTACCCTGAA	GCTGACTCCT	TCCCAAGCTG	TGCCGCTGCC	CACCGAAAGG	2640
45	GCAGCAGCAG	CAACGAGCCC	TCCTCCGACT	CCCTGAGCTC	ACCCACGCTG	CTGGCCCTGT	2700
	GAGCAGTCAG	AGAAGGCAAG	GCAGCCGGCA	TCCAGACGTG	CCACTGCCCC	AGCTGGTGCA	2760
50	TTACAGAGAG	GAGAAACACG	TCTTCCCTCG	AAGGTTCCCG	TCGACCTAGG	GAGGACCTTA	2820

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EP 0 599 077 A2

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CCTGTTTCGTG AAACACACCA GGCTGTGGGC CTCAAGGACT TGCAAGCATC CACATCTGGC 2880
CTCCAGTCCT CACCTCTTCC AGAGATGTAG CAAAAACAAA ACAAACAAA AAAAAAACCC 2940
5 GCATGGAGTG TGTGTTCCT AGTGACACCT GAGAGCTGGT AGTTAGTAGA GCATGTGAGT 3000
CAAGGCCTGG TCTGTGTCTC TTTTCTCTTT CTCCTTAGTT TTCTCATAGC ACTAACTAAT 3060
CTGTTGGGTT CATTATTGGA ATTAACCTGG TGCTGGATTG TATCTAGTGC AGCTGATTTT 3120
10 AACAATACCT ACTGTGTTCC TGGCAATAGC GTGTTCCAAT TAGAAACGAC CAATATTAAA 3180
CTAAGAAAAG ATAGGACTTT ATTTTCCAGT AGATAGAAAT CAATAGCTAT ATCCATGTAC 3240
TGTAATCCTT CAGCGTCAAT GTTCATTGTC ATGTTACTGA TCATGCATTG TCGAGGTGGT 3300
15 CTGAATGTTT TGACATTAAC AGTTTTCAT GAAACGTTT TTATTGTGTT TTCAATTTAT 3360
TTATTAAGAT GGATTCTCAG ATATTATAT TTTTATTTTA TTTTTTTCTA CCCTGAGGTC 3420
TTTCGACATG TGGAAAGTGA ATTTGAATGA AAAATTTTAA GCATTGTTTG CTTATTGTTC 3480
20 CAGGACATTG TCAATAAAAG CATTTAAGTT GAATGCGACC ACCTTCTTGC TCTCTTTATT 3540
CTCAGTTT 3548

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(2) INFORMATION FOR SEQ ID NO: 6:

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25 (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 6210 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: single
    (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: join(889..1029, 1783..2034, 2466..2573, 2688
35         ..3326)

    (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 402..453
        (D) OTHER INFORMATION: /note= "transcriptional activator
40 region"

    (ix) FEATURE:
        (A) NAME/KEY: prim_transcript
        (B) LOCATION: 734..3329

    (ix) FEATURE:
45     (A) NAME/KEY: exon
        (B) LOCATION: 889..1029
        (D) OTHER INFORMATION: /note= "c-fos protein, exon1"

    (ix) FEATURE:
50     (A) NAME/KEY: intron

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EP 0 599 077 A2

(B) LOCATION: 1030..1782
(D) OTHER INFORMATION: /note= "c-fos, intron A"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1783..2034

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 2035..2465

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2466..2573

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 2574..2687

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 2688..3329

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCAGGAACAG TGCTAGTATT GCTCGAGCCC GAGGGCTGGA GGTAGGGGA TGAAGGTCTG	60
CTTCCACGCT TTGCACTGAA TTAGGGCTAG AATTGGGGAT GGGGGTAGGG GCGCATTCCT	120
TCGGGAGCCG AGGCTTAAGT CCTCGGGGTC CTGTACTCGA TGCCGTTTCT CCTATCTCTG	180
AGCCTCAGAA CTGTCTTCAG TTTCCGTACA AGGGTAAAAA GCGCTCTCT GCCCCATCCC	240
CCCCGACCTC GGAACAAGG GTCCGCATTG AACCAGGTGC GAATGTTCTC TCTCATTCTG	300
CGCCGTTCCC GCCTCCCCTC CCCAGCCGC GGCCCCGCC TCCCCCGCA CTGCACCCTC	360
GGTGTGGCT GCAGCCCGCG AGCAGTCCC GTCAATCCCT CCCCCTTAC ACAGGATGTC	420
CATATTAGGA CATCTGCGTC AGCAGGTTT CACGGCCTTT CCCTGTAGCC CTGGGGGAG	480
CCATCCCCGA AACCCTCAT CTTGGGGGCG CCACGAGACC TCTGAGACAG GAACTGCGAA	540
ATGCTCACGA GATTAGGACA CGCGCCAAGG CGGGGCGAGG GAGCTGCGAG CGCTGGGGAC	600
GCAGCCGGGC GGCCGAGAA GCGCCAGGC CCGCGGCCA CCCCTCTGGC GCCACCGTGG	660
TTGAGCCCGT GACGTTTACA CTCATTATA AAACGCTTGT TATAAAAGCA GTGGCTGCGG	720
CGCCTCGTAC TCCAACCGCA TCTGCAGCGA GCAACTGAGA AGCCAAGACT GAGCCGCGCG	780
CCGCGGCGCA GCGAACGAGC AGTGACCGTG CTCCTACCA GCTCTGCTTC ACAGCGCCCA	840
CCTGTCTCCG CCCCTCGGCC CCTCGCCCG CTTGCCTAA CCGCCACG ATG ATG TTC	897
Met Met Phe	
1	

EP 0 599 077 A2

	TCG GGC TTC AAC GCA GAC TAC GAG GCG TCA TCC TCC CGC TGC AGC AGC	945
	Ser Gly Phe Asn Ala Asp Tyr Glu Ala Ser Ser Ser Arg Cys Ser Ser	
	5 10 15	
5	GCG TCC CCG GCC GGG GAT AGC CTC TCT TAC TAC CAC TCA CCC GCA GAC	993
	Ala Ser Pro Ala Gly Asp Ser Leu Ser Tyr Tyr His Ser Pro Ala Asp	
	20 25 30 35	
	TCC TTC TCC AGC ATG GGC TCG CCT GTC AAC GCG CAG GTAAGGCTGG	1039
	Ser Phe Ser Ser Met Gly Ser Pro Val Asn Ala Gln	
10	40 45	
	CTTCCCCTCG CCGCGGGGCC GGGGGCTTGG GGTCGCGGAG GAGGAGACAC CGGGCGGGAC	1099
	GCTCCAGTAG ATGAGTAGGG GGCTCCCTTG TGCCTGGAGG GAGGCTGCCG TGGCCGGAGC	1159
15	GGTGCCGGCT CGGGGGCTCG GGA CTGCTC TGAGCGCAG CACGCTTGCC ATAGTAAGAA	1219
	TTGGTTCCCC CTTCGGGAGG CAGGTTTCGTT CTGAGCAACC TCTGGTCTGC ACTCCAGGAC	1279
	GGATCTCTGA CATTAGCTGG AGCAGACGTG TCCCAAGCAC AAACCTCGCTA ACTAGAGCCT	1339
20	GGCTTCTTCG GGGAGGTGGC AGAAAGCGGC AATCCCCCCT CCCCCGGCAG CCTGGAGCAC	1399
	GGAGGAGGGA TGAGGGAGGA GGGTGCAGCG GGCGGGTGTG TAAGGCAGTT TCATTGATAA	1459
	AAAGCGAGTT CAT TCTGGAG ACTCCGGAGC GGCGCCTGCG TCAGCGCAGA CGTCAGGGAT	1519
25	ATTTATAACA AACCCCTTT CAAGCAAGTG ATGCTGAAGG GATAACGGGA ACGCAGCGGC	1579
	AGGATGGAAG AGACAGGCAC TGCGCTGCGG AATGCCTGGG AGGAAAAGGG GGAGACCTTT	1639
	CATCCAGGAT GAGGGACATT TAAGATGAAA TGTCCGTGGC AGGATCGTTT CTCTTCACTG	1699
30	CTGCATGCGG CACTGGGAAC TCGCCCCACC TGTGTCCGGA ACCTGCTCGC TCACGTCGGC	1759
	TTTCCCCTTC TGT TTTGTTT TAG GAC TTC TGC ACG GAC CTG GCC GTC TCC	1809
	Asp Phe Cys Thr Asp Leu Ala Val Ser	
	50 55	
35	AGT GCC AAC TTC ATT CCC ACG GTC ACT GCC ATC TCG ACC AGT CCG GAC	1857
	Ser Ala Asn Phe Ile Pro Thr Val Thr Ala Ile Ser Thr Ser Pro Asp	
	60 65 70	
	CTG CAG TGG CTG GTG CAG CCC GCC CTC GTC TCC TCT GTG GCC CCA TCG	1905
	Leu Gln Trp Leu Val Gln Pro Ala Leu Val Ser Ser Val Ala Pro Ser	
40	75 80 85	
	CAG ACC AGA GCC CCT CAC CCT TTC GGA GTC CCC GCC CCC TCC GCT GGG	1953
	Gln Thr Arg Ala Pro His Pro Phe Gly Val Pro Ala Pro Ser Ala Gly	
	90 95 100	
45	GCT TAC TCC AGG GCT GGC GTT GTG AAG ACC ATG ACA GGA GGC CGA GCG	2001
	Ala Tyr Ser Arg Ala Gly Val Val Lys Thr Met Thr Gly Gly Arg Ala	
	105 110 115 120	
	CAG AGC ATT GGC AGG AGG GGC AAG GTG GAA CAG GTGAGGAACT CTAGCGTACT	2054
	Gln Ser Ile Gly Arg Arg Gly Lys Val Glu Gln	
50	125 130	
55		

EP 0 599 077 A2

	CTTCCTGGGA ATGTGGGGGC TGGGTGGGAA GCAGCCCCGG AGATGCAGGA GCCCAGTACA	2114
	GAGGATGAAG CCACTGATGG GGCTGGCTGC ACATCCGTAA CTGGGAGCCC TGGCTCCAAG	2174
5	CCCATTCCAT CCCAACTCAG ACTCTGAGTC TCACCCTAAG AAGTACTCTC ATAGTTTCTT	2234
	CCCTAAGTTT CTTACCGCAT GCTTTCAGAC TGGGCTCTTC TTTGTTCTCT TGCTGAGGAT	2294
	CTTATTTTAA ATGCAAGTCA CACCTATTCT GCAACTGCAG GTCAGAAATG GTTTCACAGT	2354
10	GGGGTGCCAG GAAGCAGGGA AGCTGCAGGA GCCAGTTCTA CTGGGGTGGG TGAATGGAGG	2414
	TGATGGCAGA CACTTTTACT GAATGTCGGT CTTTTTTTGT GATTATTCTA G TTA TCT Leu Ser	2471
15	CCA GAA GAA GAA GAG AAA AGG AGA ATC CGA AGG GAA AGG AAT AAG ATG Pro Glu Glu Glu Glu Lys Arg Arg Ile Arg Arg Glu Arg Asn Lys Met 135 140 145	2519
	GCT GCA GCC AAA TGC CGC AAC CGG AGG AGG GAG CTG ACT GAT ACA CTC Ala Ala Ala Lys Cys Arg Asn Arg Arg Arg Glu Leu Thr Asp Thr Leu 150 155 160 165	2567
20	CAA GCG GTAGGTACTC TGTGGGTTGC TCCTTTTAA AACTTAAGGG AAAGTTGGAG Gln Ala	2623
25	ATTGAGCATA AGGGCCCTTG AGTAAGACTG TGTCTTATGC TTTCCTTTAT CCCTCTGTAT	2683
	ACAG GAG ACA GAC CAA CTA GAA GAT GAG AAG TCT GCT TTG CAG ACC GAG Glu Thr Asp Gln Leu Glu Asp Glu Lys Ser Ala Leu Gln Thr Glu 170 175 180	2732
30	ATT GCC AAC CTG CTG AAG GAG AAG GAA AAA CTA GAG TTC ATC CTG GCA Ile Ala Asn Leu Leu Lys Glu Lys Glu Lys Leu Glu Phe Ile Leu Ala 185 190 195	2780
	GCT CAC CGA CCT GCC TGC AAG ATC CCT GAT GAC CTG GGC TTC CCA GAA Ala His Arg Pro Ala Cys Lys Ile Pro Asp Asp Leu Gly Phe Pro Glu 200 205 210	2828
35	GAG ATG TCT GTG GCT TCC CTT GAT CTG ACT GGG GGC CTG CCA GAG GTT Glu Met Ser Val Ala Ser Leu Asp Leu Thr Gly Gly Leu Pro Glu Val 215 220 225 230	2876
40	GCC ACC CCG GAG TCT GAG GAG GCC TTC ACC CTG CCT CTC CTC AAT GAC Ala Thr Pro Glu Ser Glu Glu Ala Phe Thr Leu Pro Leu Leu Asn Asp 235 240 245	2924
	CCT GAG CCC AAG CCC TCA GTG GAA CCT GTC AAG AGC ATC AGC AGC ATG Pro Glu Pro Lys Pro Ser Val Glu Pro Val Lys Ser Ile Ser Ser Met 250 255 260	2972
45	GAG CTG AAG ACC GAG CCC TTT GAT GAC TTC CTG TTC CCA GCA TCA TCC Glu Leu Lys Thr Glu Pro Phe Asp Asp Phe Leu Phe Pro Ala Ser Ser 265 270 275	3020

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EP 0 599 077 A2

	AGG CCC AGT GGC TCT GAG ACA GCC CGC TCC GTG CCA GAC ATG GAC CTA	3068
	Arg Pro Ser Gly Ser Glu Thr Ala Arg Ser Val Pro Asp Met Asp Leu	
	280 285 290	
5	TCT GGG TCC TTC TAT GCA GCA GAC TGG GAG CCT CTG CAC AGT GGC TCC	3116
	Ser Gly Ser Phe Tyr Ala Ala Asp Trp Glu Pro Leu His Ser Gly Ser	
	295 300 305 310	
	CTG GGG ATG GGG CCC ATG GCC ACA GAG CTG GAG CCC CTG TGC ACT CCG	3164
	Leu Gly Met Gly Pro Met Ala Thr Glu Leu Glu Pro Leu Cys Thr Pro	
10	315 320 325	
	GTG GTC ACC TGT ACT CCC AGC TGC ACT GCT TAC ACG TCT TCC TTC GTC	3212
	Val Val Thr Cys Thr Pro Ser Cys Thr Ala Tyr Thr Ser Ser Phe Val	
	330 335 340	
15	TTC ACC TAC CCC GAG GCT GAC TCC TTC CCC AGC TGT GCA GCT GCC CAC	3260
	Phe Thr Tyr Pro Glu Ala Asp Ser Phe Pro Ser Cys Ala Ala Ala His	
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	Arg Lys Gly Ser Ser Ser Asn Glu Pro Ser Ser Asp Ser Leu Ser Ser	
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	CCC ACG CTG CTG GCC CTG TGAGGGGGCA GGGAAGGGGA GGCAGCCGGC	3356
	Pro Thr Leu Leu Ala Leu	
	375 380	
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	GAGGGTTTCCT GTAGACCTAG GGAGGACCTT ATCTGTGCGT GAAACACACC AGGCTGTGGG	3476
	CCTCAAGGAC TTGAAAGCAT CCATGTGTGG ACTCAAGTCC TTACCTCTTC CGGAGATGTA	3536
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	ATTAATAAT CTATTGGGTT CATTATTGGA ATTAACCTGG TGCTGGATAT TTTCAAATTG	3716
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	TTTTATTGTG TTTTAAATTT ATTTATTAAG ATGGATTCTC AGATATTTAT ATTTTATTTT	4016
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	CAACCTTGTG CTCTTTTCAT TCTGGAAGTC TTGTAAGTTT CTGAAAGGTA TTATTGGAGA	4196
	CCAGTTTGTC AAGAAGGGTA GCTGCTGGAG GGGGACACAC CCTCTGTCTG ATCCCTTATC	4256
50	AAAGAGGACA AGGAACTAT AGAGCTGATT TTAGAATATT TTACAAATAC ATGCCTTCCA	4316

EP 0 599 077 A2

	TTGGAATGCT	AAGATTTTCT	ACTGCTTCTG	GGGACGGGAA	ACCGCTGTGT	AACAGCTTTT	4376
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	TTTGTACAGA	CTATTTTAAC	TTTTTATTCT	CACAAAATCA	CGTGGAGGGT	TATTCTACTT	4556
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	CCTAGCTACT	TGAGAGGCTG	AGACAGGAAA	ATCACTTGAA	CTCGGGAGGC	GGATGTTGCA	4916
	GCGAACTGAG	ATTGCGCCAT	TGCACTCCAG	CCTGGGCAAC	AAGATTGAAA	CTCTGTTTAA	4976
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	CATATGCCCT	TCATTCAAGT	TATAGTGGGC	GGAAGTGGGG	GAGAAAAAGT	TGCTCAGAAA	5156
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	ATCACTCCTA	TATCACACAC	CACTTGGGGT	GCTGTTTCTG	CTAGACTTGT	GATGACAGTG	5696
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	AAACTAAATA	CAGAATTAAT	ATAACTAACA	AACACCTGGT	CTGAAATAAC	AAGGTGATCT	5816
	ACCCTGGAAG	GAACCCAGCT	GGTGGGCCAG	GAGCGGTGGC	TCACACCTGT	AATCCAGCA	5876
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	ACATGGCAAA	ACCCAGTGTG	CTTCTGTTGT	CCCAGCTACA	CTACTCAGGA	GGCTGAGGCA	5996
50	GGAGTATGAC	TTGAGCCTGG	GAGGGGGAGG	TTGCAGAGAA	CTGATATTGC	ACCACCACTG	6056
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EP 0 599 077 A2

CACTCCAGCC TGGGTGACAC AGCAAAACCC TATCTCAAAA AAAAAAAAAA AAAAAAGGAA 6116
 CCCAGCTGGT TCCTGTAGGT GTGCAATAAT AACAAACCAGA GGAAGAAAAG GAAGACGATT 6176
 5 TCCCAGATGA AGAAGGGCAG CTGGACCTTC GGAC 6210

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
 10 (A) LENGTH: 380 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met	Met	Phe	Ser	Gly	Phe	Asn	Ala	Asp	Tyr	Glu	Ala	Ser	Ser	Ser	Arg
1				5					10					15	
Cys	Ser	Ser	Ala	Ser	Pro	Ala	Gly	Asp	Ser	Leu	Ser	Tyr	Tyr	His	Ser
			20					25					30		
Pro	Ala	Asp	Ser	Phe	Ser	Ser	Met	Gly	Ser	Pro	Val	Asn	Ala	Gln	Asp
		35					40					45			
Phe	Cys	Thr	Asp	Leu	Ala	Val	Ser	Ser	Ala	Asn	Phe	Ile	Pro	Thr	Val
	50					55					60				
Thr	Ala	Ile	Ser	Thr	Ser	Pro	Asp	Leu	Gln	Trp	Leu	Val	Gln	Pro	Ala
	65				70					75					80
Leu	Val	Ser	Ser	Val	Ala	Pro	Ser	Gln	Thr	Arg	Ala	Pro	His	Pro	Phe
				85					90				95		
Gly	Val	Pro	Ala	Pro	Ser	Ala	Gly	Ala	Tyr	Ser	Arg	Ala	Gly	Val	Val
			100					105					110		
Lys	Thr	Met	Thr	Gly	Gly	Arg	Ala	Gln	Ser	Ile	Gly	Arg	Arg	Gly	Lys
		115				120						125			
Val	Glu	Gln	Leu	Ser	Pro	Glu	Glu	Glu	Glu	Lys	Arg	Arg	Ile	Arg	Arg
	130					135					140				
Glu	Arg	Asn	Lys	Met	Ala	Ala	Ala	Lys	Cys	Arg	Asn	Arg	Arg	Arg	Glu
	145				150					155					160
Leu	Thr	Asp	Thr	Leu	Gln	Ala	Glu	Thr	Asp	Gln	Leu	Glu	Asp	Glu	Lys
				165					170					175	
Ser	Ala	Leu	Gln	Thr	Glu	Ile	Ala	Asn	Leu	Leu	Lys	Glu	Lys	Glu	Lys
			180					185					190		
Leu	Glu	Phe	Ile	Leu	Ala	Ala	His	Arg	Pro	Ala	Cys	Lys	Ile	Pro	Asp
		195					200					205			
Asp	Leu	Gly	Phe	Pro	Glu	Glu	Met	Ser	Val	Ala	Ser	Leu	Asp	Leu	Thr
	210					215					220				

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Gly Gly Leu Pro Glu Val Ala Thr Pro Glu Ser Glu Glu Ala Phe Thr
 225 230 235 240
 Leu Pro Leu Leu Asn Asp Pro Glu Pro Lys Pro Ser Val Glu Pro Val
 5 245 250 255
 Lys Ser Ile Ser Ser Met Glu Leu Lys Thr Glu Pro Phe Asp Asp Phe
 260 265 270
 Leu Phe Pro Ala Ser Ser Arg Pro Ser Gly Ser Glu Thr Ala Arg Ser
 10 275 280 285
 Val Pro Asp Met Asp Leu Ser Gly Ser Phe Tyr Ala Ala Asp Trp Glu
 290 295 300
 Pro Leu His Ser Gly Ser Leu Gly Met Gly Pro Met Ala Thr Glu Leu
 15 305 310 315 320
 Glu Pro Leu Cys Thr Pro Val Val Thr Cys Thr Pro Ser Cys Thr Ala
 325 330 335
 Tyr Thr Ser Ser Phe Val Phe Thr Tyr Pro Glu Ala Asp Ser Phe Pro
 20 340 345 350
 Ser Cys Ala Ala Ala His Arg Lys Gly Ser Ser Ser Asn Glu Pro Ser
 355 360 365
 Ser Asp Ser Leu Ser Ser Pro Thr Leu Leu Ala Leu
 25 370 375 380

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
 30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGCAGTGAGG TCAGGGGTGG GGAAGCCCAG GGCTGGGGAT TCCCCATCT

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40 Claims

1. Anti-tumor cellular vaccine comprising tumor cells into which *c-fos* gene (SEQ ID No:6) alone or together with *c-jun* gene (SEQ ID No:3) have been inserted.
2. An anti-tumor vaccine according to claim 1 wherein said tumor cells are human tumor cells.
3. An anti-tumor vaccine according to claim 1 or 2 wherein said tumor cells are derived from tumor cells having metastatic competence.
4. An anti-tumor vaccine according to any of claims 1 to 3 comprising human tumor cells transfected with *c-fos* gene.
5. An anti-tumor vaccine according to any of claims 1 to 3 comprising human tumor cells transfected with both *c-fos* and *c-jun* genes.
6. An anti-tumor vaccine according to claim 5 wherein the *c-fos* and *c-jun* genes have been introduced into said tumor cells on a single expression vector enabling constitutive production of the *c-fos* and *c-jun* gene products *in vivo*.

7. An anti-tumor vaccine according to claim 5 wherein the *c-fos* and *c-jun* genes have been introduced into said tumor cells on different expression vectors enabling constitutive production of the *c-fos* and *c-jun* gene products *in vivo*.
- 5 8. An anti-tumor vaccine according to claim 4 wherein *c-fos* gene has been introduced into said tumor cells on an expression vector enabling constitutive production of the *c-fos* gene product *in vivo*.
9. An anti-tumor vaccine according to any of claims 1 to 8 wherein said tumor cells are cells from a tumor from the individual to be vaccinated.
- 10 10. An anti-tumor vaccine according to any of claims 1 to 8 wherein said tumor cells are derived from individuals other than the individual to be vaccinated.
11. An anti-tumor vaccine according to any of claims 1 to 8 comprising transfected tumor cells which show
15 increased levels of expression of MHC class I protein.
12. An anti-tumor vaccine according to any of claims 1 to 11 wherein said tumor cells have been inactivated.
- 20 13. An anti-tumor vaccine according to claim 12 wherein said tumor cells have been inactivated by treatment with gamma or X-rays and/or mitomycin C.
14. An anti-tumor vaccine according to any of claims 1 to 13 comprising from about 1×10^6 to about 1×10^9 transfected tumor cells.
- 25 15. An anti-tumor vaccine according to any of claims 1 to 14 characterized in that it is formulated as an injection.
16. An anti-tumor vaccine comprising antigens expressed by *c-fos* gene alone or together with *c-jun* genes.
- 30 17. An anti-tumor vaccine according to any of claims 1 to 16 for the treatment of a patient suffering from cancer to prevent and/or inhibit the development of metastases.
18. A method for the production of an anti-tumor vaccine according to any of claims 1 to 16 comprising the
35 steps of:
 - a) removing cells from a primary tumor of the patient by biopsy or surgery;
 - b) dispersing the cells in a medium;
 - c) inserting into said cells a vector comprising the human *c-fos* gene or the human *c-fos* and *c-jun* genes;
 - 40 d) optionally selecting the positive transfectants that show high expression of *c-fos* and MHC class I genes; and
 - e) inactivating the transfectants by gamma- or X-ray irradiation and/or treatment with mitomycin C.
19. A method of inducing MHC expression in tumor cells from endogenous genes by transfecting the tumor
45 cells with *c-fos* or *c-fos* and *c-jun* genes.

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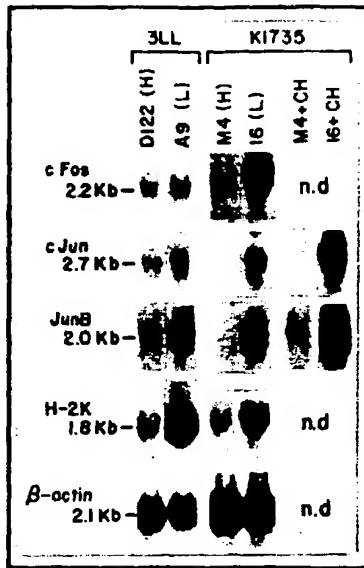


Fig-1A

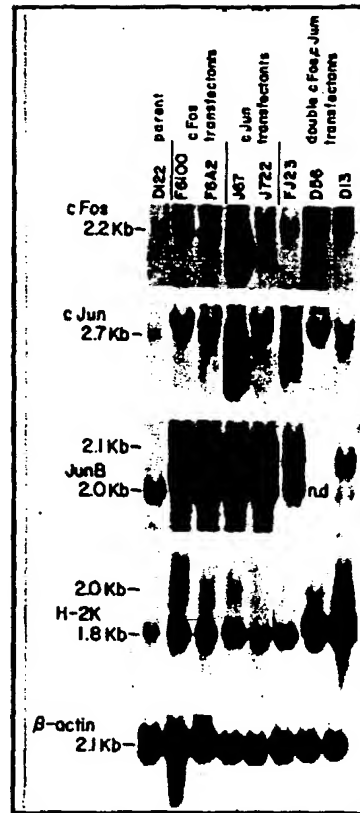


Fig-1B

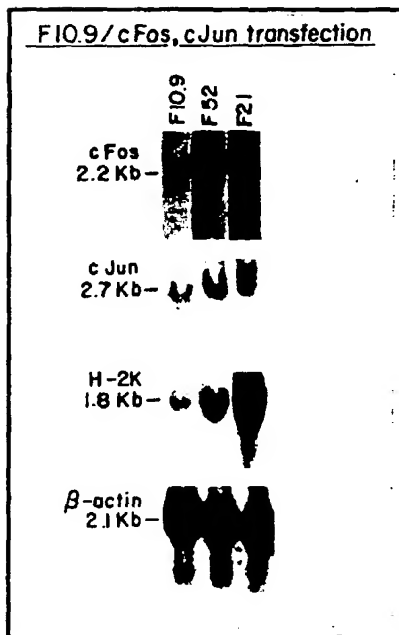


Fig-1C

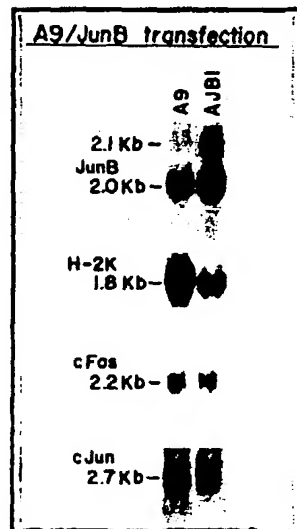


Fig-1D

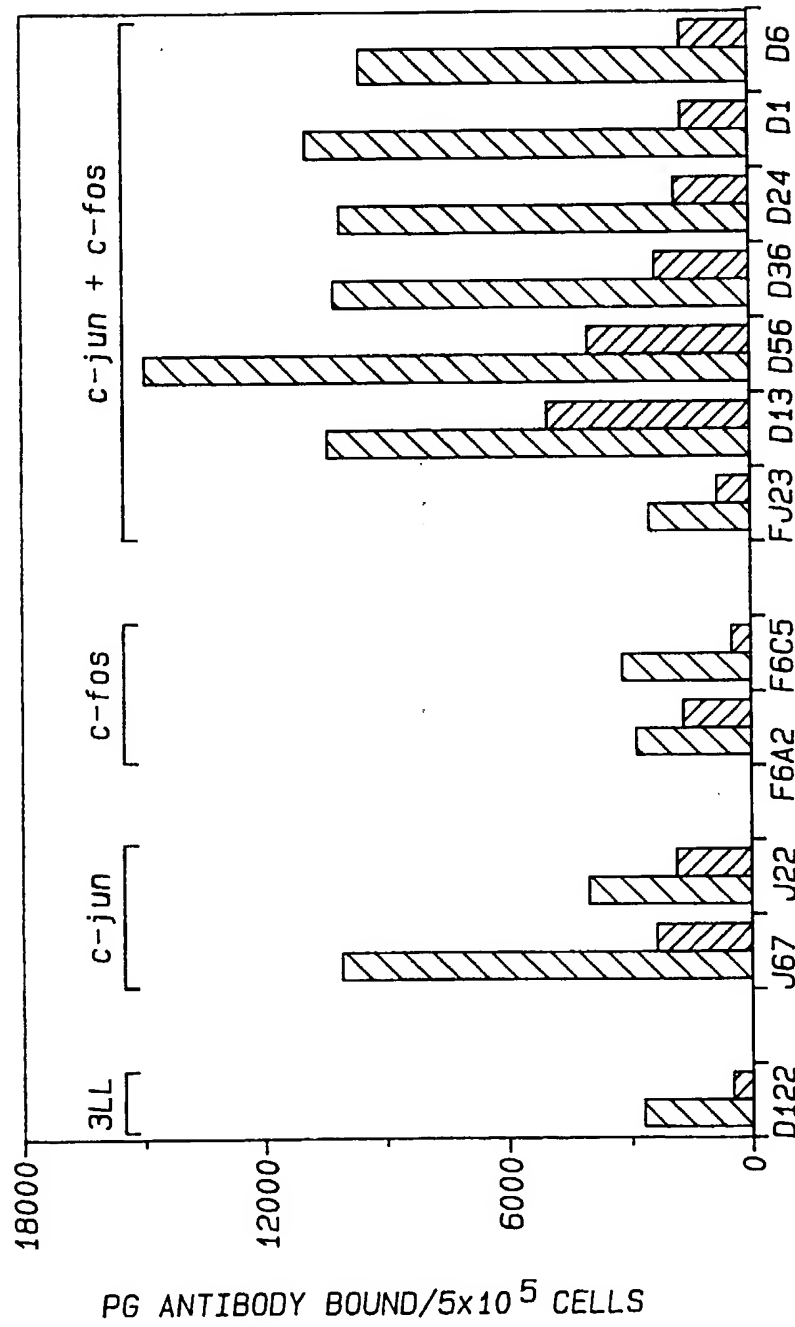
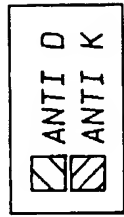


Fig-2A

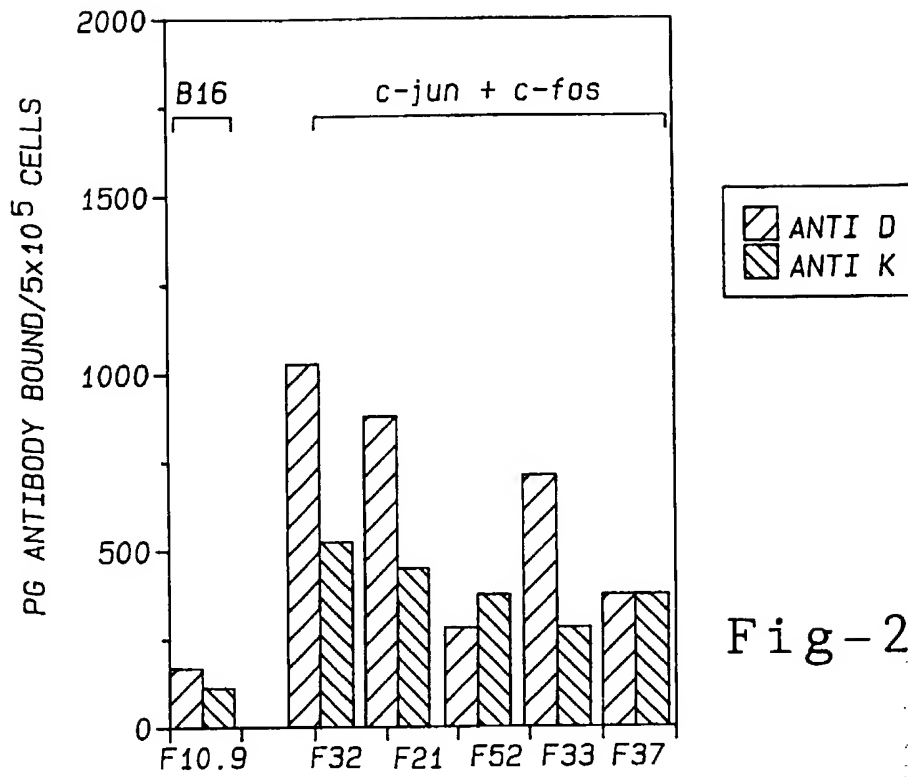


Fig-2B

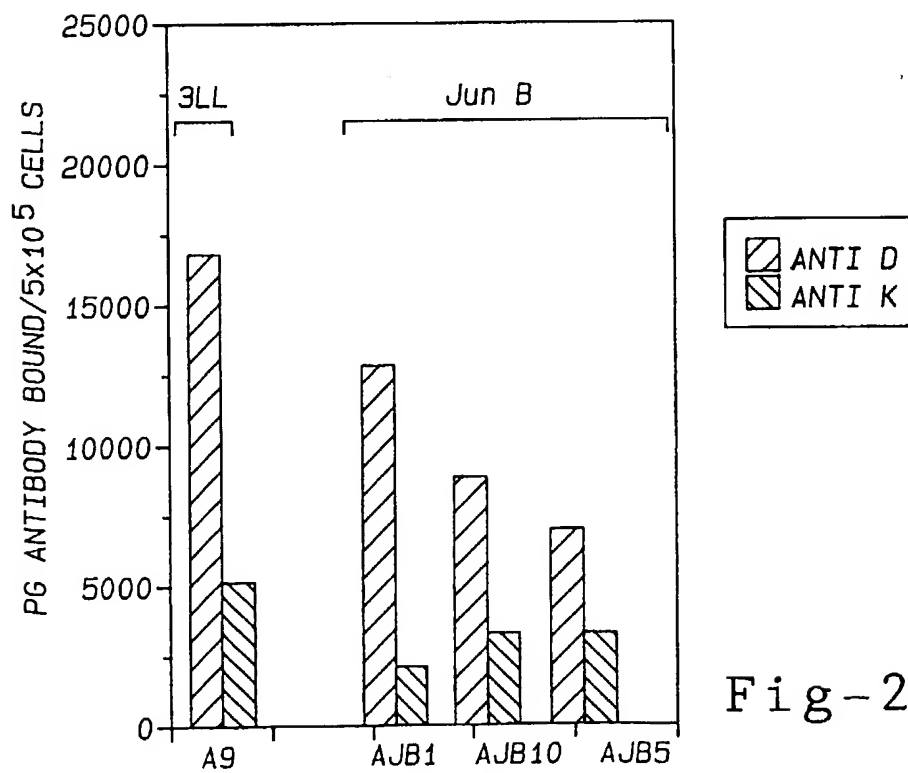


Fig-2C

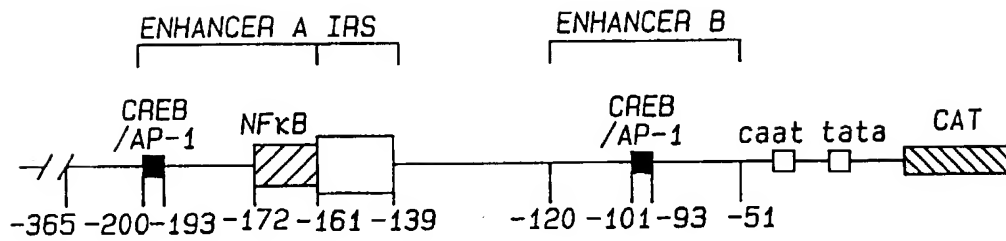


Fig-3A

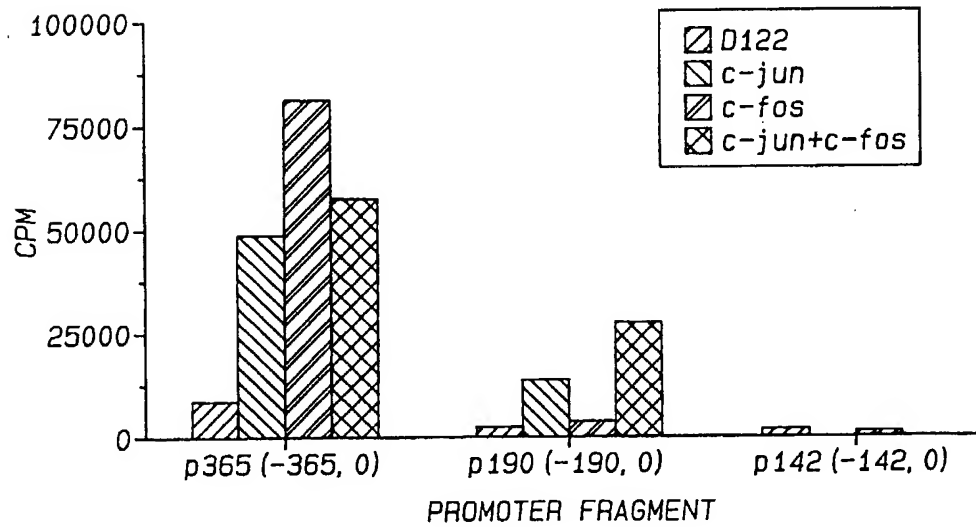


Fig-3B

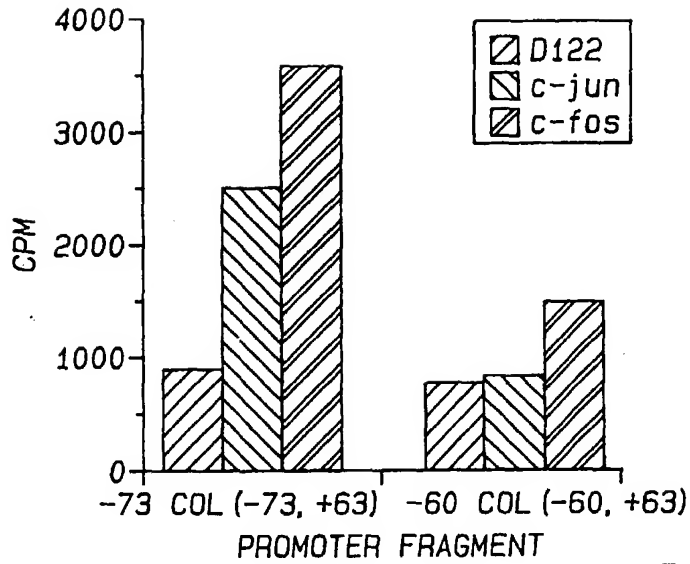


Fig-3C

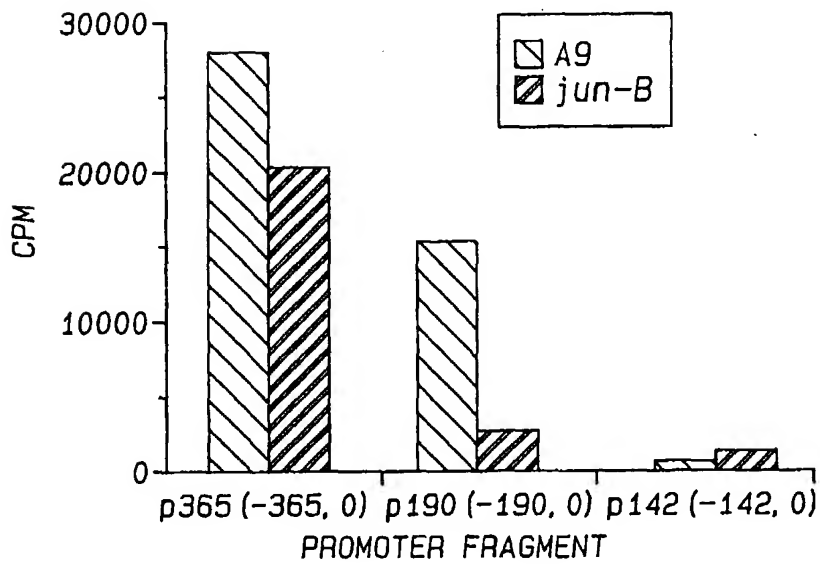


Fig-3D

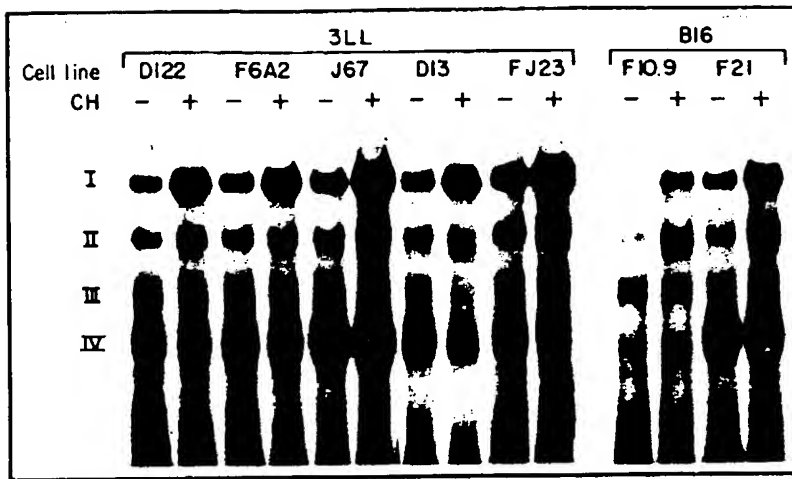


Fig-4A

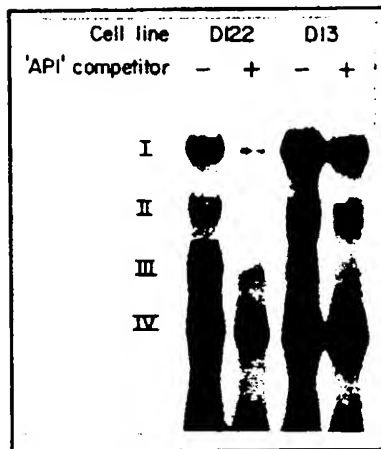
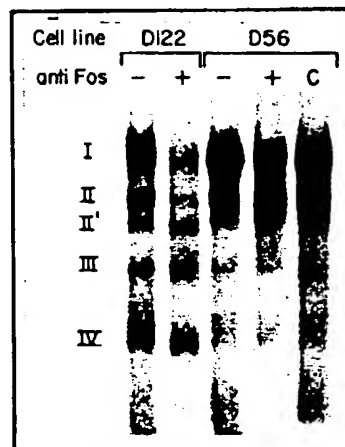
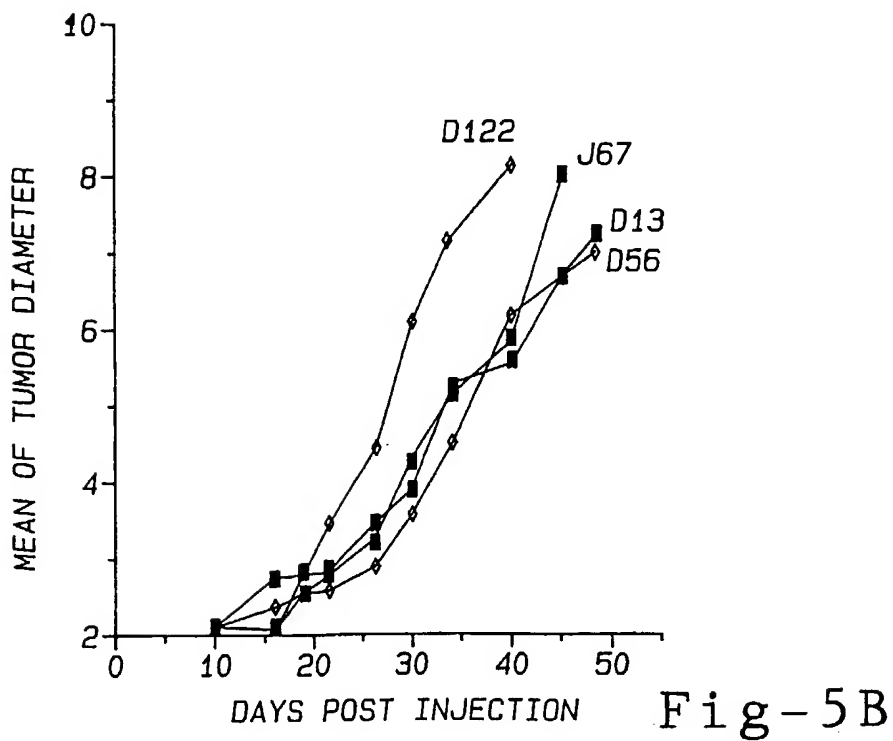
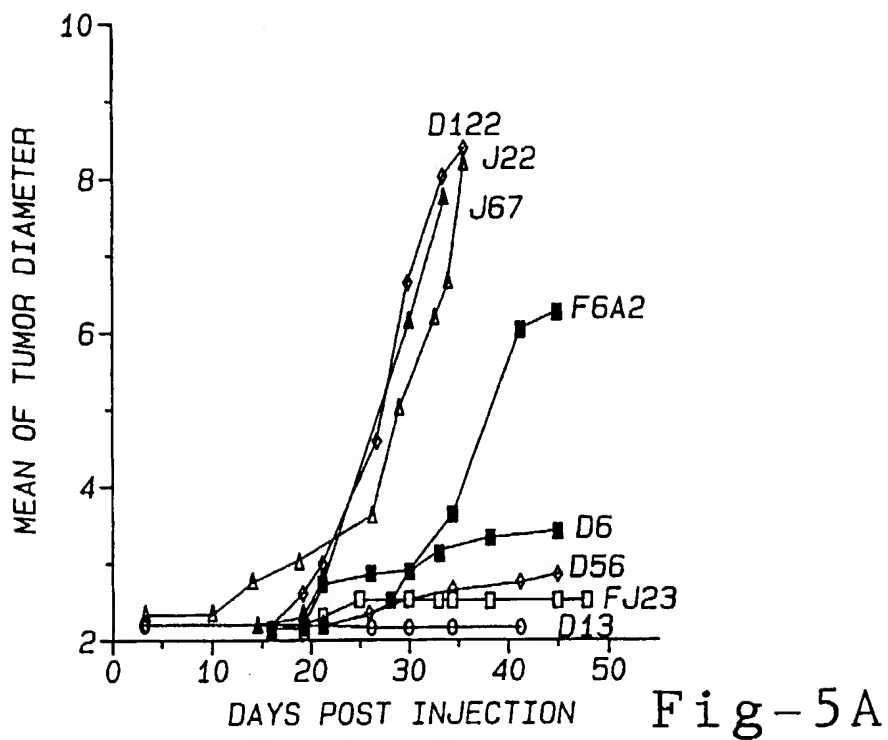


Fig-4B

Fig-4C





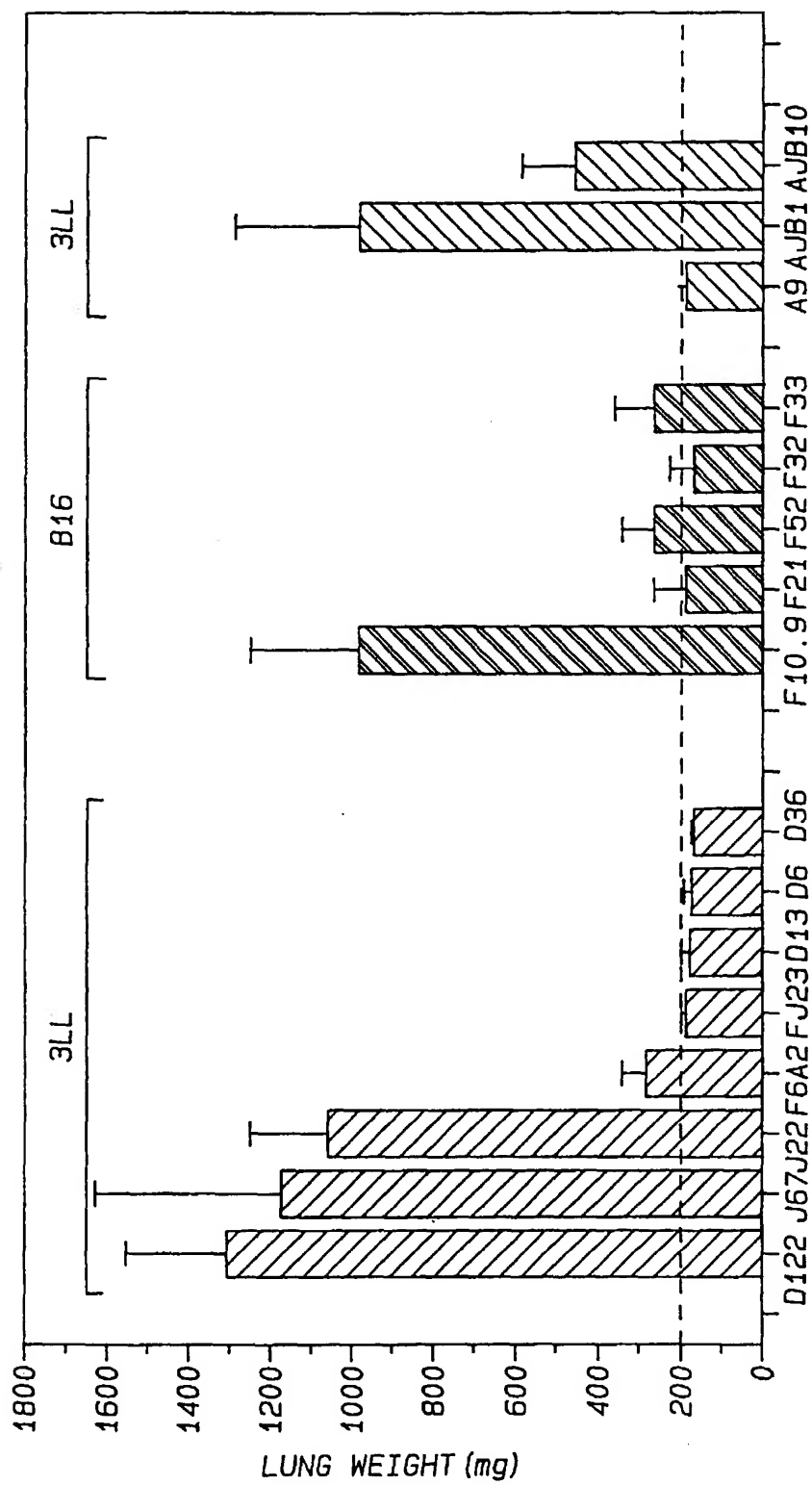


Fig-5C

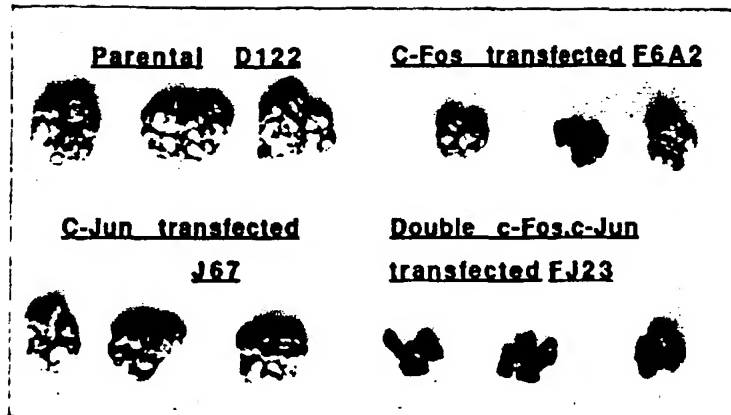


Fig--6A

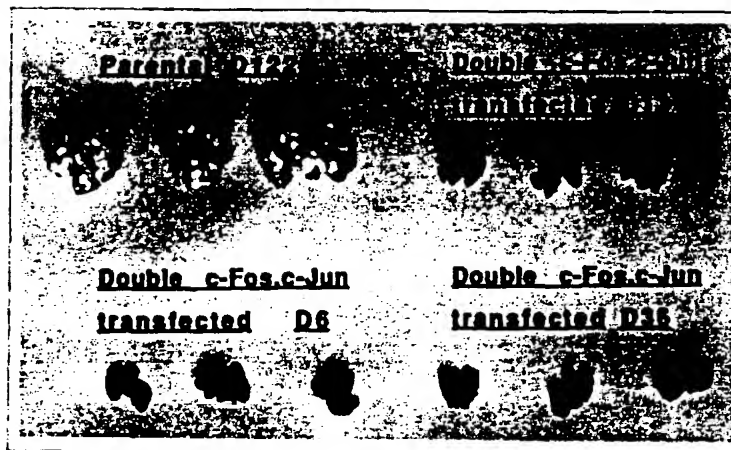


Fig--6B

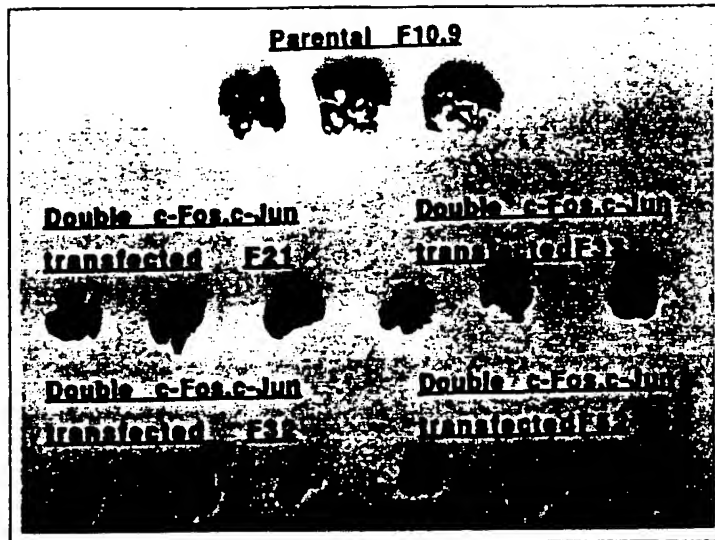


Fig-6C

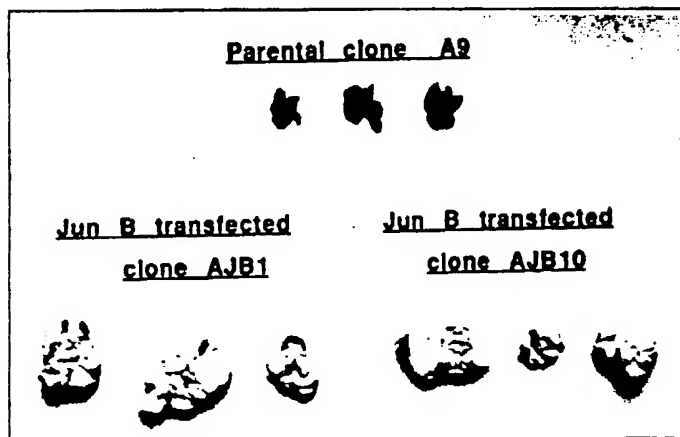


Fig-6D

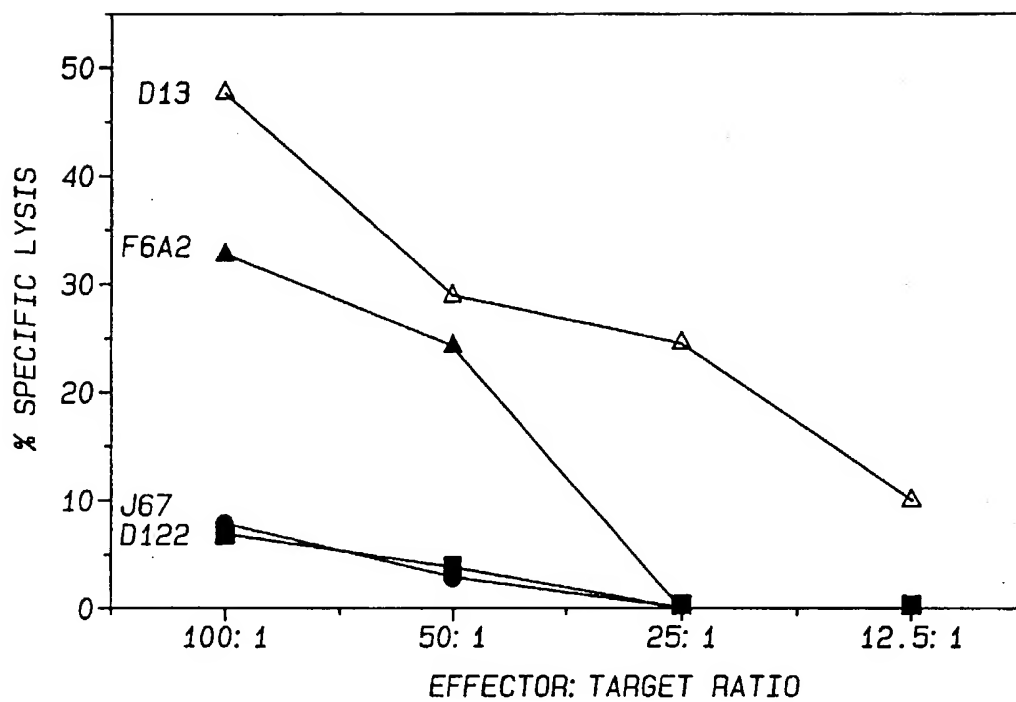


Fig-7